

REPVIAS

RESEARCH & PRACTICE in VETERINARY & ANIMAL SCIENCE

Volume: 2 Issue: 1 Year: 2025

e-ISSN: 3023-6681



RESEARCH & PRACTICE in VETERINARY & ANIMAL SCIENCE

Volume: 2, Issue: 1 (January 2025)

International Peer Reviewed Journal

Owner

Necmettin Erbakan University

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Necmettin Erbakan University, Faculty of Veterinary Medicine,
Ereğli, Konya, Türkiye

Publication Type

Periodical

Publication Period

Published twice-annual (January and July)

Print Date

January 2025

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Orhaniye Quarter University Street No:15, Ereğli, Konya, Türkiye

Phone: 90 332 777 00 66 / 7231

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ISSN: 3023-6681

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Residue and Efficiency Testing in Flash-Acting Amitraz Fumigation

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Article Info

Received: 11.06.2024
Accepted: 19.08.2024
Online First: 04.12.2024
Published: 23.01.2025

Keywords:

Amitraz,
Apis mellifera,
Efficacy,
Flash-fumigation,
Varroa destructor.

ABSTRACT

Due to global climate change, more attention needs to be paid to the control of some parasites. One of these, *Varroa destructor*, a honey bee parasite, is a pest that requires regular monitoring and effective treatment. At this point, healthy bees are vital for pollination and sustainable biodiversity. Honeybee colonies face threats like diseases and *Varroa* mite infestations. Beekeepers use acaricides such as amitraz for control. In flash-amitraz treatment trials on 14 mite-infected colonies, application resulted 95.1% antiparasitic effectiveness, with honey residues below MRL. Honey sampled from the colonies was tested for residues at the National Veterinary Reference Laboratory in the Etlik, Ankara. The reference laboratory conducted an analysis of amitraz residues utilizing the techniques of gas chromatography-mass spectrometry. Through this process, the laboratory was able to examine and identify these residues with the use of advanced GC-MS technology. Mite infestation level, antiparasitic efficiency and side effects of treatment were tested. The therapeutic efficacy of amitraz was evaluated using the Henderson-Tilton formula. This formula was employed as the method to effectively assess how well amitraz performs in treatment. The formulation and application of flash-amitraz are crucial for safety and effectiveness. Flash fumigation offers effective mite control while maintaining residue levels near the MRL, making it a reliable method for managing *Varroa destructor* in honey production. Amitraz-based acaricides are licensed veterinary preparations available in various commercial forms and remain widely preferred worldwide. Antiparasitic drug resistance poses a significant threat to bee health and colony productivity. Residue-free foods are crucial for global food safety and consumer health, particularly within the "One Health One Medicine" framework.

To cite this article:

Muz, M.N., & Özdemir, N. (2025) Residue and Efficiency Testing in Flash-Acting Amitraz Fumigation. *Research and Practice in Veterinary and Animal Science (REPVAS)*, 2(1), 1-10.
<https://doi.org/10.69990/REPVAS.2025.2.1.1>

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INTRODUCTION

Honeybee breeding is an option against global hunger enabling rural people to sustain themselves participating in local production. Bees are also crucial pollinators and key driver of socioeconomic stability and environmentally sustainability. Recent research reveals alarming decline rates in honeybee colonies, raising serious concerns about the long-term implications for biodiversity conservation (Patel et al., 2021). The World Organisation for Animal Health (WOAH), formerly known as the OIE, has established control measures for the trade and movement of bees to prevent the introduction of new bee diseases into the territories of importing countries. These measures are detailed in the WOAH (OIE) Terrestrial Animal Health Code. Varroosis, a disease caused by the Varroa mite, is one of the six classified bee diseases included in this code. By adhering to the guidelines set forth by the WOAH, countries can effectively mitigate the risk of spreading bee diseases, such as Varroosis, through international trade and movement of bees, ultimately safeguarding the health of their bee populations and the sustainability of their apiculture industries (Fanelli & Tizzani, 2020). The epizootic ectoparasite mite Varroa destructor causes losses in Western honeybee (*Apis mellifera*) colonies. Mite-born viral infections reveal clinical symptoms called parasitic mite syndrome. Beekeepers must need an efficient Varroosis treatment against viral epidemics, colony collapses and irregular swarms (More et al., 2017).

Beekeepers need effective and residue-free miticides that should be non-toxic for bee colonies. If government authorities deem it necessary, they may impose additional restrictions or regulate the sale and use of registered pesticides within the country due to concerns regarding resistance, efficacy, toxicity and residue issues (Bubnic et al, 2021; EPA, 2020).

The amitraz, coumaphos, flumethrine, tau-fluvalinate, and other active substances have been registered as varroocides. Therapeutics including licenced chemical synthetics may result antiparasitic drug resistance risk, diminished efficacy and residue issues if not strictly applied as directed by the manufacturer's instructions (Almecija et al., 2022).

Amitraz, in the formamidine class, is the only acaricide chemical that blocks octopamine receptors in the mite CNS. Amitraz decomposes into volatiles during flash-fumigation. The three primary metabolites of amitraz in honey degrade within maximum ten days [N-(2,4-dimethyl phenyl) N'-methyl formamidine (DPMF), N-2,4-dimethyl phenyl formamide (DMF), and 2,4-dimethylaniline (2,4-DMA)] (Gupta, 2018).

Various fumigation approaches have own unique ways of exterminating ectoparasites whether ranging from gassy treatments to solid and liquid applications (Stejskal et al., 2021). The risk of residue accumulation in fumigation depends on the application process, chemical formulation, matrices and other conditions (Almecija et al., 2022). Unlike water-insoluble amitraz, the high solubility of many other synthetic varroocides in honey has resulted in cumulative residue problems.

As per EU regulations (EC) No 396/2005 and 2017/623, the accepted amitraz residue level in honey and bee products is 200 µg/kg (Efsa, 2016). At this point, the lower limit (the sum of all metabolites containing amitraz and 2,4-dimethylaniline group) as the Amitraz MRL standard by the Reference Laboratories of the Ministry of Agriculture and Food in Turkey has been accepted as 200 µg/kg in honey (Http link: Official Gazzette Türkiye, 2017).

Certain treatments can also disrupt honeybees' physiological behavioural processes on colony level (O'Neal et al., 2017). Licenced Amitraz flash treatment (gas fumigation) which consists of seven smokes (Rulamit-VA), minimizes the risk of residue due to the active ingredient with negligible water solubility due to instant (only two minutes) fumigant exposure and has not been reported honeybee toxicity. So, we complemented test honey amitraz residue, acaricide efficiency and check the flash-

fumigation if the bee life-threatening, to aiding beekeepers in choosing effective veterinary medicines.

MATERIALS and METHODS

The research was performed in a stationary apiary located in Sarkoy, Tekirdag, Türkiye after honey harvest where on 40° 47' 50" N 27° 21' 56" E, in August 2018. The amitraz flash fumigation trials were conducted in four-teen naturally mite-infested colonies (four colonies as control trials and ten for served as treatment).

Detection of Infestation Level

Colony infestation levels were evaluated before and after treatment using the powdered sugar test to count mites. The test was performed one hour before and after four-day flash-fumigation treatment process. First, the number of bees in each sample was accurately counted. Then, the infestation level (IL) was calculated using the formula: $IL = (VN / BN) \times 100$, where VN represents the number of Varroa destructor mites and BN represents the total number of bees in the sample. This standardized procedure ensures consistent and accurate assessment of infestation levels across all samples. The acaricide's effectiveness was also evaluated using this method. This standardized protocol guarantees a consistent and accurate evaluation of infestation levels across all specimens, allowing for the assessment of the acaricide's efficacy (Bava et al., 2022, Pietropaoli et al. 2021).

Flash-fumigation Treatment

The amitraz-based flash fumigation treatment uses impregnated cardboard plates measuring 20 x 10 cm, each containing 265 mg of the active ingredient, to treat up to 10 bee colonies. The treatment is highly effective, as the cardboard burns without a flame upon ignition, rapidly releasing amitraz.

Colonies were fumigation treated four times with an interval of three days to test antiparasitic effectiveness. The "Varroa destructor" samples fall-off to the bottom board were counted after an hour of each amitraz fumigation treatment. Since there was no ant issue in the apiary the sticky paper was not needed.

The application of Rulamit-VA is comparable to the use of a traditional beekeeper's smoker. Notably, the treatment only targets phoretic mites on adult bees, as the fumes cannot penetrate wax capping. When used in a well-ventilated colony, as directed in the product manual, the flash fumigation process utilizing amitraz-impregnated cartons leaves no residual odours, residues, or hazardous substances.

Flash-fumigation was performed using a bee smoker with a "fume blow tip." (Figure). The hive's flight hole was fully open during the amitraz fumigation. Colonies began wing ventilating inside the hive after the first fumigation. Seven flash fumes were administered consecutively through the flight hole of 12 hives, completing the each of process only in a minute. The bees were not negatively affected by the seven flash fumes. The treatment was repeated four times, with three-day intervals. After fumigation, Varroa mites that fell off were collected from the sticky bottom board after an hour. Treatment efficacy is determined by a predefined formula. Four colonies are used as the control group (Bava et al., 2022).

Honey Sampling and Residue Analysis

Sealed and unsealed honeycombs were sampled before and after the end treatment for residue analysis. Each flash fumigation was trialled in four tours, with an interval of three days (seven fanning of the application smoke). All honey samples were analysed for amitraz and its metabolites [N-(2,4-dimethyl phenyl) N'-methyl formamidine (DPMF), N-2,4-dimethyl phenyl formamide (DMF), and 2,4-dimethylaniline (2,4-DMA)] for the MRL. Honey samples were analyzed using GC-MS at the TÜRKAK - accredited "National Reference Laboratory" The Veterinary Control Center Research Institute, Etlik, Ankara.

Figure. Bee hive smoker



RESULTS

This study investigated the metabolite markers of amitraz residues in honey when honeybee colonies were treated with Rulamit-VA, examining within antiparasitic efficacy. During treatment, no colony-level issues or symptomatic observations regarding honeybee health were reported. The Henderson-Tilton (Henderson and Tilton, 1955) formula calculated the miticide efficacy of the GMP-certified licensed amitraz-containing fumigant Rulamit-VA to be 95.1%.

Due to amitraz's well-documented instability in honey, specialized analytical methods were employed to determine total residues of amitraz, including its metabolites. The residue levels of amitraz and its metabolites in sealed and unsealed comb honey samples were found to be well below the reference Maximum Residue Limit (MRL). Amitraz marker residues did not exceed one-tenth of the MRL level in honey samples.

DISCUSSION

While the latest research on amitraz in the current world literature reveals the reliability of amitraz, it has been determined that there is a gap in this regard in Turkey. The commercial product named Rulamit-VA (265 mg amitraz included) which is the subject of this research, is a locally produced veterinary medical preparation subject to prescription, licensed by the Ministry of Agriculture and Food of the Republic of Turkey. Contrary to Aydin and Girisgin's previous publications (Aydin and Girisgin, 2010) in which they preferred imported Amitraz products with foreign commercial formulations now it is preferred to investigate a completely domestic amitraz product.

Global honey production reached 1,830,768 tonnes in 2022, with Türkiye contributing 118,292 tons. The honey is deemed unfit for human consumption and commerce if residues exceed the Maximum

Residue Limit (MRL). Most synthetic acaricides exhibit lipophilic properties, leading them to accumulate predominantly in beeswax. When it comes to beeswax, amitraz has a unique characteristic - it breaks down completely within 24 hours, making it the "only licensed" unstable acaricide in this matrix. On the other hand, all synthetic lipophilic acaricides are relatively stable in beeswax and their concentration increases with each additional treatment and wax recycling (Kast et al., 2021, Medici et al., 2015).

Unfortunately, the persistent presence of acaricides in the wax contributes to developing acaricide-resistant Varroa mites. On the contrary of wax, acaricides to leave residues in honey, they must possess water-soluble characteristics. Consequently, the water-insoluble acaricide origin residue risk is comparatively very low, often falling too below the established MRLs in honey (Gupta, 2018).

The parasitic mite known as Varroa destructor poses the gravest threat to honeybee populations among all the factors. A severe infestation by Varroa mites can lead to significant colony mortality within a few weeks in honeybee colonies that receive insufficient treatment. Amitraz employs a mite-specific mode of action, avoiding the induction of detoxification gene expression in honeybees (Boncristiani et al., 2012). Amitraz stands out as the only acaricide sensitive to acidic environments. Since honey is acidic, amitraz breaks down when mixed with honey. Moreover, if used in accordance with the package insert, it decomposes entirely within ten days, posing no risk threats to the brood or the end consumers (Korta et al., 2001).

A US-based study published in 2022 examined the residual levels of amitraz and its metabolites in honey and beeswax after administering a dose of Apivar (amitraz strip) five times higher than normal. Amitraz itself was not detected in any bee products after 42 days. However, trace amounts of its metabolites DMPF and DMA were found in samples taken 28 days after treatment. These findings show that even at high doses, amitraz metabolite residues in bee products remain within acceptable limits (Chaimanee et al., 2021).

Beekeepers often purchase amitraz-containing strips in bulk at a low cost, leaving them in the hive for extended periods, hoping for improved results. However, these strips have a shelf life of 24 months from the manufacturing date, and once opened, their potency decreases significantly within 1-2 weeks due to amitraz oxidation, leading to diminished effectiveness. When metabolized, amitraz binds to neuron receptors, modifying the behaviours of mites. This alteration prevents mites from interacting with bees, consequently halting the reproduction of Varroa destructor. Notably, amitraz is metabolized at a rate 7,000 times higher in V. destructor than in honeybees. Multiple amitraz-based treatments are commercially available, they vary in their galenic formulations and efficacy (Almecija et al., 2024). Amitraz has been found to have low acute toxicity to honeybee larvae, with an LC50 of 461.4 mg/L in a study (Dai et al., 2017). Another study showed that even with a four-day exposure, Amitraz had low toxicity and did not impact their survival rate. Products approved for honeybee use are safe if the packaging instructions are followed (Dai et al., 2018).

Unlike long-acting registered pyrethroids, flash fume of amitraz fumigation can't linger as long in the hive environment, resulting in lower residue levels and less bee exposure. As exposure diminishes, the development of resistance progresses at a more gradual pace. Water-insoluble amitraz has distinct metabolomics from pyrethroids, and commercial formulations like fast-acting flash fumes cause tardy resistance among Varroa populations. These characteristics minimize the risk of toxic effects on bees and reduce the potential risk to human consumers of hive products. Three registered active ingredients, (Coumaphos, flumethrin and tau-fluvalinate) have been found to remain stable in honey for approximately nine months, while the risk is considerably lower for flash-acting amitraz (Bischoff and

Moiseff, 2023; Bubnič et al. 2021).

Amitraz, a varroa mite pesticide used in Spain since 1999, has maintained almost 100% effectiveness despite long-term use and has not shown any significant resistance development. In this context, updated new regulations in the USA allow Amitraz at a concentration of 3.33% to alleviate varroa mite infestations, minimize hive product residues and prioritize consumer welfare.

In a study conducted with Apivar strip formulation in Spain in 2015, the effectiveness of long acting amitraz strip was found to be 64.3% (Leza et al. 2015).

Researchers tried the same strips in Türkiye, declared that they found its effectiveness to be 99.43% (Aydin and Girişgin, 2010). Similarly, Adjlane and Haddad, who tried the same strips in 2017, found the effectiveness to be 39.23% (Adjlane and Haddad, 2017).

It is not known exactly how much the resistance genes expressed against amitraz in Varroa destructor are affected by commercial formulations of amitraz and similar reasons such as application errors, seasonal and management differences (Hernández-Rodríguez et al., 2022; Marsky et al., 2024).

The use of smouldering Amitraz tablets like Apiwarol in Poland (for brief durations (20-30 minutes) carries a minimal risk of residual traces. However, an analysis of honey samples collected from areas exposed to the smoke directly, including the brood chambers and supers, revealed only negligible residue accumulation, indicating a low potential for contamination (Pohorecka et al., 2018). A study conducted in Türkiye reported the effectiveness of amitraz fumigation as 30% (Girisgin et al, 2019).

Fumigation is a technique that involves using a range of different chemicals to eradicate and effectively rid an environment of pests and insects. The long-acting/slow-released amitraz strips (42-56 days) are acts in different. Solid fumigation of licenced Apiwarol tablet acts during short-term smouldering (20 minutes) treatment (Pohorecka et al., 2018; Semkiw et al., 2013) however the non-flammable carton-impregnated amitraz flash-fumigation acts via snap gaseous matrix (only a minute).

CONCLUSION

In conclusion, Ensuring the health of honeybees is crucial for combating global hunger, enhancing agricultural pollination, and promoting rural development. Only products that prioritize food safety should be employed to support bee populations.

Ethical Approval

The authors state that; Insects, being invertebrate animals, are not subject to the ethical considerations that typically require approval from ethics committees for research involving vertebrates.

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Data Collection (CRediT 2) Author 1 (%50) – Author 2 (%50)

Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%50) – Author 2 (%50)

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Funding

The authors state that there is not any funding in this study.

Conflict of Interest

The authors state that there is no conflict of interest between the authors.

Sustainable Development Goals (SDG)

3 Good Health and Well-Being

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The Example of Çankırı Province in Combating Brucellosis in Cattle

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Article Info

Received: 17.05.2024
Accepted: 20.08.2024
Online First: 04.12.2024
Published: 23.01.2025

Keywords:

B. abortus S19,
Bovine brucellosis,
Conjunctival vaccination,
Control,
Fight.

ABSTRACT

Brucellosis in bovines is an infectious disease that leads to significant health, productivity, and economic losses. It is also a zoonotic disease, posing a serious threat to public health. This disease is widespread in Türkiye as well as globally. Vaccination is a crucial and cost-effective measure, with the most commonly utilized vaccine strains worldwide for bovine brucellosis control being *B. abortus* S19 and RB51. In this study, the results of the intensive efforts to combat bovine brucellosis between 2011 and 2015 in Çankırı province, as well as the results of the mass *B. abortus* S19 conjunctival vaccination campaigns carried out within the scope of the Ministry project in 2012-2013, are evaluated. Within the scope of the research, retrospective data obtained from various public institutions affiliated with the ministry were evaluated. The results of disease control, eradication and vaccination studies carried out in the field were evaluated epidemiologically within the framework of legal regulations. As a result of close monitoring of bovine brucellosis outbreaks and mass conjunctival vaccination in Çankırı province, the number of outbreaks has decreased more than four times compared to the average in Türkiye. Additionally, there is a 95-fold reduction in the number of animals compensated for the disease and a more than 200-fold decrease in the total amount of compensation paid. This study examines the efforts to control bovine brucellosis in Çankırı province, particularly focusing on mass conjunctival vaccination campaigns using the *B. abortus* S19 vaccine strain. Given the conditions in Türkiye, the *B. abortus* S19 vaccine strain is currently deemed the most effective for preventing brucellosis in bovines. Ultimately, to attain the planned objectives in bovine brucellosis control in Türkiye, the study concludes that vaccination during a suitable period, which does not pose a risk to pregnancy and ensures robust herd immunity, is the optimal approach.

To cite this article:

Çakır, Ş., & Karataş Yeni, D. (2025). *The Example of Çankırı Province in Combating Brucellosis in Cattle*. *Research and Practice in Veterinary and Animal Science (REPVAS)*, 2(1), 11-21.

<https://doi.org/10.69990/REPVAS.2025.2.1.2>

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INTRODUCTION

Bovine brucellosis is a contagious and infectious disease that is usually caused by *Brucella abortus* (*B. abortus*) and results in abortion, premature calf birth, infertility, and productivity losses in cattle (Peker et al., 2010; Inlamea et al., 2016; Khurana et al., 2021; Rahimnahal et al., 2023). Bovine brucellosis, which is also a zoonotic disease, is also an important cause of serious public health problems and economic losses (Güzelant et al., 2009; Ulaş et al., 2012; Khurana et al., 2021; Rahimnahal et al., 2023). *B. abortus* is one of the most common agents in bovine and has been classified as a category (B) pathogen with the potential to be used as a biological weapon (Peker et al., 2010; Khurana et al., 2021). Except for brucellosis-free countries, it is widespread worldwide and its prevalence is high especially in developing countries in the Middle East, Central Asia, and the Mediterranean (Güzelant et al., 2009; Peker et al., 2010; Inlamea et al., 2016; Kepenek Kurt et al., 2021). Brucellosis in Türkiye is particularly prevalent in the Central, Eastern, and Southeastern Anatolia Regions. It is a zoonotic disease that is widespread in our country and persists as an ongoing public health concern (Demir and Orhan, 2012; Sayar et al., 2019; Kepenek Kurt et al., 2021).

Brucellosis is recognized by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) as the most common zoonotic disease worldwide (Khurana et al., 2021; de Oliveira et al., 2022). Laine et al. (2023) reported that the annual incidence of human brucellosis worldwide is approximately 1.6-2.1 million new cases, according to their calculations (Laine et al., 2023). Infected animals are the main source of infection in humans. Transmission to humans usually occurs through the consumption of unpasteurized milk or dairy products obtained from infected animals (especially raw milk, fresh cheese, butter, etc.) (Ulaş et al., 2012; Sayar et al., 2019; Kepenek Kurt et al., 2021). The disease is more commonly observed in rural areas among livestock breeders, veterinary health workers, butchers, slaughterhouse and milkers, and scientists. Bovine brucellosis is also referred to as contagious abortion or Bang's disease (Peker et al., 2010; Khurana et al., 2021). The primary clinical symptoms observed in humans include shaking, high fever, weakness, as well as muscle and joint pains (Demir and Orhan, 2012). An effective vaccine has not yet been developed to protect people from brucellosis (Güzelant et al., 2009). Currently, vaccinating animals, pasteurizing milk, and taking measures to prevent contact with infected animal products and materials are the most effective methods for preventing transmission and protecting humans from brucellosis infection (Peker et al., 2010).

In addition to affecting public health, bovine brucellosis also affects the health of animals, reducing their productivity and causing significant economic losses in the livestock sector (Rahimnahal et al., 2023). The disease tends to spread rapidly within the infected herd, leading to a decrease in milk and meat production, as well as occurrences of abortions, stillbirths or premature births, and reproductive losses such as infertility (de Oliveira et al., 2022). The economic importance attributed to the disease is based on direct losses from abortions, loss of offspring, infertility, weight loss, decrease in milk production, and inhibition of trade in animals and their products (Dorneles et al. 2015). This disease also has negative socioeconomic effects, including a decrease in calf birth rate due to prolonged calving intervals, increased costs for purchasing re-breeding animals, diminished competitiveness in the market, treatment expenses, and loss of labor (Inlamea et al., 2016). It has been reported that the rate of abortion in susceptible herds varies between 30 and 80%. *Brucella*-positive animals are one of the major causes of the spread of brucellosis (Khurana et al., 2021). Due to the serious medical and socioeconomic consequences of the disease, it is important to prevent the transmission of the infection through vaccination of animals (Schurig et al., 2002).

Vaccination is the main measure to control bovine brucellosis. The most widely used vaccine strains in the world are *B. abortus* S19 and RB51 (Dorneles et al., 2015; de Oliveira et al., 2022). Both vaccines are effective in preventing abortion and infection, as well as providing long-term protection (Dorneles et al. 2015). The RB51 vaccine was developed in 1982 and is a rough rifampicin-resistant strain of *B. abortus* biovar 1 that does not express the O-side chain lipopolysaccharide in its membrane. Therefore, this vaccine does not stimulate antibodies detected by routine serological tests. There is no serological test available to detect RB51 infection (de Oliveira et al., 2022). Since the Brucella RB51 strain can pass into milk in animals, it can be transmitted to humans in this way and cause infections. Its protective effect in bovine is similar to that induced by strain S19. The most commonly used vaccine to prevent bovine brucellosis is *B. abortus* strain 19 (Schurig et al., 2002). This vaccine, which has been used since 1941, is a smooth-attenuated strain of *B. abortus* biovar 1 that induces an antibody response that is indistinguishable from that induced by infection (de Oliveira et al., 2022). While S19 is an older vaccine compared to RB51 and offers longer-lasting protection (over 10 years), it remains highly effective and cost-efficient, making it widely utilized (de Oliveira et al., 2022). Additionally, *B. abortus* S19 may offer cross-protection against *B. melitensis* (Khurana et al., 2021). Currently, the *B. abortus* S19 vaccine is considered the best for preventing brucellosis in cattle, but this vaccine, which contains dead and/or attenuated live strains, may have potential side effects. The most effective and cost-efficient method for controlling the disease is through rapid and accurate diagnosis coupled with appropriate animal vaccination programs (Rahimnahal et al., 2023).

This study discusses the efforts undertaken by the Ministry of Agriculture and Forestry (MoAF) and the Çankırı Provincial Directorate of Agriculture and Forestry to fight bovine brucellosis infection between 2011 and 2015, as well as the mass conjunctival vaccination studies conducted during 2012-2013. Our study is based on retrospective field data. The aim is to effectively monitor and control epidemics by creating strong herd immunity through *B. abortus* S19 conjunctival vaccination, and to produce successful results by preventing losses. For this purpose, the efficacy and results of *B. abortus* S19 conjunctival vaccine were evaluated in Çankırı province. The research results are intended to illuminate the ongoing efforts to fight the disease and to provide guidance for decision-makers.

MATERIAL and METHOD

In this study, data from different institutions were used. The basic data of the research were taken from the records kept in the fight to control bovine brucellosis infection between 2011-2015 and the mass conjunctival vaccination studies applied with *B. abortus* S19 strain in 2012-2013 of MoAF, Çankırı Provincial Directorate of Agriculture and Forestry. According to this data, Çankırı province consists of 12 districts, including the central district. In these districts, there were a total of 9,327 cattle breeding premises in 2012-2013. The number of bovines in Çankırı province, which is the subject of the research in these years, are 133,010 and 130,658 heads, respectively. In these two years, it was determined that Çankırı province had 0.96% and 0.91% of Türkiye's overall bovine population, respectively.

The number of bovine brucellosis outbreaks in this period, the number of animals subject to conditions slaughtered due to the disease and the amount of compensation paid, the number of cattle within the scope of the "*Conjunctival Vaccine Control and Eradication of Brucella Project*", the vaccination program and the vaccination figures applied were compiled retrospectively. In addition, the bovine brucellosis outbreak numbers reported by MoAF to the World Organization for Animal Health (WOAH) during this period were used.

Another data of the research is the total number of bovines throughout Türkiye. This data was taken from the 2012-2013 animal numbers of statistics kept by the Turkish Statistical Institute (TSI).

According to TSI data in 2012-2013, when mass conjunctival vaccination studies were carried out in the fight against the disease, the number of bovines in Türkiye was 13,914,912 and 14,415,257 heads, respectively.

Bovine brucellosis is designated as a notifiable disease in accordance with the “*Regulation on Notifiable Animal Diseases and Notification*” (Official Gazette (OG) dated 22.01.2011 and numbered 27,823) published under the auspices of Law No. 5996 on “*Veterinary Services, Plant Health, Food and Feed Law*” (OG dated 13.06.2010 and numbered 27,610). Currently, no treatment is provided for this disease. Furthermore, the “*Regulation on Fighting Brucellosis*” (OG dated 03.04.2009 and numbered 27,189) falls under the purview of Law No. 5996. Presently, efforts to combat this disease are conducted in accordance with the provisions outlined in this regulation, as well as the circular pertaining to the “*Fighting Animal Diseases and Control of Animal Movements*”, which undergoes annual renewal.

The “*Regulation on Compensated Animal Diseases and Compensation Rates*” (OG dated 14.01.2012 and numbered 28,173) and “*Regulation on Compensation for Animal Diseases*” (OG dated 06.03.2013 and numbered 28,579), under the scope of Law No. 5996 have been published. According to these regulations, if bovine animals are found to be infected with brucellosis through serological or bacteriological examination, they are required to be slaughtered subject to the condition or culled, with compensation paid at a rate of 9/10 of their appraised value.

The vaccine used for bovine brucellosis, under the trade name Brupen A (5-10x10⁹ colony-forming units (CFU) for 1 dose of vaccine (50 µl)), was produced by the İstanbul/Pendik Veterinary Control Institute Directorate affiliated with the MoAF.

This epidemiological study involves analyzing data from the mentioned institutions, considering relevant legal regulations, and reviewing the outcomes of disease control and vaccination efforts conducted in the field.

Since retrospective data were used in this study, there was no need to obtain ethical permission. However, since bovine brucellosis is a notifiable disease, the necessary legal permission was obtained from MoAF, General Directorate of Food and Control (GDFC) to publish the research results. In addition, statements of consent were received from the relevant people for the images used in Figure 2.

RESULTS

Outbreak Numbers

Çankırı is a province located in the Central Anatolia Region. It is a settlement with restrictions for cattle breeding due to its geographical structure and pasture situation. Türkiye has a share of less than 1% of bovine assets. However, as can be seen detailed in Table, when we compare the number of bovine brucellosis outbreaks in Çankırı province and Türkiye in general, 57/486 (11.73%) in 2011 and 101/1.696 (5.96%) in 2012 were reported, respectively. In 2010, upon the notification of bovine brucellosis outbreaks density, on-site inspections were carried out in Çankırı Provincial Directorate of Agriculture and Forestry, Animal Health Branch Directorate and 11 District Directorates. Information on the subject was provided at the meetings attended by the provincial director, district directors, veterinary health workers and all other stakeholders. In these meetings, coordination was ensured between the teams and actions were taken in coordination with MoAF, GDFC. Within the possibilities, the personnel, document, and equipment deficiencies of the teams were eliminated. Existing equipment

was maintained and used in disinfection works. Since 2011, disease emergence and extinctions of transferred and newly notified outbreaks have been closely monitored. The premises where the disease is extinguished, and their surroundings are disinfected with sprayers. In 2012, a mass conjunctival vaccination program was initiated by MoAF within the scope of the "*Conjunctival Vaccine Control and Eradication of Brucella Project*". As the details are given below, the bovine included in the program were vaccinated with a high percentage rate. As a result of all this fight against bovine brucellosis disease, there has been a significant decrease in the number of disease outbreaks over the years. In this context, in 2013, 2014 and 2015, the number of disease outbreaks reported in Çankırı was 30/1,319 (2.27%), 11/596 (1.85%) and 9/315 (2.85%), respectively, compared to Türkiye.

Table. Number of bovine brucellosis outbreaks in Türkiye and Çankırı province (MoAF, WOA)

YEARS	ÇANKIRI PROVINCE	TÜRKİYE
2011	57	486
2012	101	1,696
2013	30	1,319
2014	11	596
2015	9	315

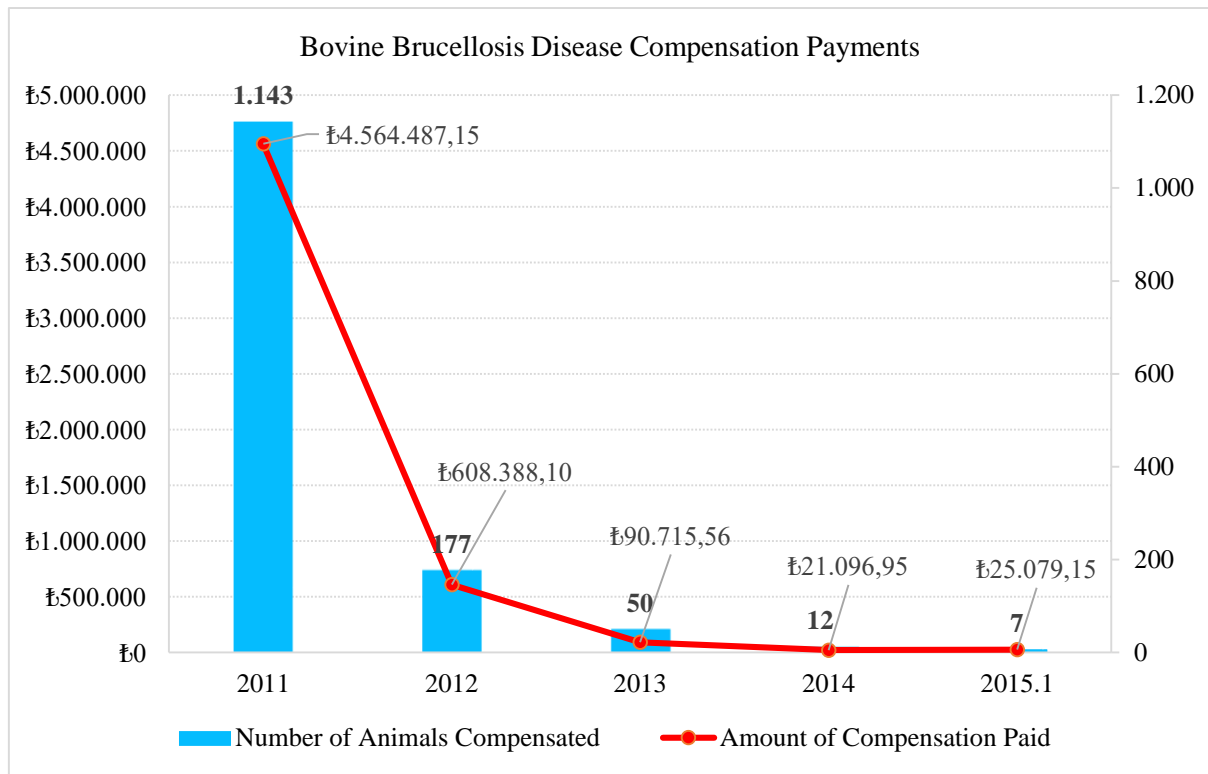
Compensation Payments

In this context, an intense fight has been carried out in Çankırı province since 2011. The animals that were detected as positive in the premises where the disease emergence was sent to conditional slaughter or culled. A compensation payment of 4,564,487.15 ₺ was made for 1,143 bovines, mainly due to bovine brucellosis in 2011. According to the information obtained, this figure corresponded to approximately quarter of the compensation paid for bovine brucellosis in Türkiye. As a result of the fight carried out and mass conjunctival vaccination studies, there has been a significant decrease in the number of animals culled or slaughtered subject to the condition and the amount of compensation paid, as the details can be seen in Figure 1. In 2012, 2013, 2014, and the first half of 2015, the number of bovines affected by bovine brucellosis and the corresponding compensation paid were 177 bovines (608,388.10 ₺), 50 bovines (90,715.56 ₺), 12 bovines (21,096.95 ₺), and 7 bovines (25,079.15 ₺), respectively. In the 4.5-year period (2011-2015.1) covered in this research, a total of 1,389 bovine were sent to conditional slaughter or culled due to bovine brucellosis. For these animals, a total of 5,309,766.91 ₺ compensation was paid to the breeders after deducting the roasting cost (Figure 1).

Vaccination Program

According to the final report of the project "*Eradication of Brucellosis and Tuberculosis in Türkiye*" conducted through collaboration between Türkiye and the Netherlands in 2012, the individual prevalence of brucellosis in bovines was reported as 2.6%, while the herd prevalence was reported as 6.9% (Bartels et al., 2012). Prior to 2012, as part of the program aimed at fighting bovine brucellosis, the *B. abortus* S19 vaccine was administered subcutaneously to female calves aged between 4 and 8 months.

Figure 1. Number of animals compensated, and amount of compensation paid due to bovine brucellosis in Çankırı province (2011-2015.1*) (₺: Turkish lira) (MoAF)



*Based on the first six months data in 2015.

With the consent of the MoAF authority dated 31.12.2011, the "Project for Control and Eradication of *Brucella* with Conjunctival Vaccine" (Circular No: 2012/03) came into effect in 2012. Implemented within this framework, the project aimed to initially reduce the prevalence of brucella in bovine herds to less than 1%, followed by the complete eradication of the disease through the test and slaughter method. According to the project guidelines, starting from 2012, it was planned to administer the *B. abortus* S19 conjunctival vaccine to all female bovines twice, with an interval of 12 months. In subsequent years, the aim was to vaccinate adult female bovines that could not be vaccinated previously, as well as female calves aged between 3 and 6 months, thereby implementing a 10-year vaccination program.

In 2012, out of the 133,010 bovines in Çankırı province, 85,000 were enrolled in the vaccination program by MoAF. Throughout the province, vaccination was administered to 81,773 bovines, achieving a significantly high vaccination rate of 96.2% (Figure 2). In 2013, 95,750 out of 130,658 bovines were included in the vaccination program. Across the province, 79,031 bovines were vaccinated, resulting in a vaccination rate of 82.5%. In subsequent years, the program continued by vaccinating previously unvaccinated animals and female calves aged 3-6 months, based on vaccine availability. Furthermore, an informational brochure about brucellosis disease and the importance of vaccination was prepared and distributed to breeders and their organizations. The distribution of vaccines sent as part of the program throughout the province and the number of vaccinations administered by the teams were regularly monitored and reported to the MoAF, GDFC.

Figure 2. Çankırı province bovine brucellosis mass conjunctival vaccination studies (MoAF)



DISCUSSION

The prevalence of bovine brucellosis is notably high in Mediterranean countries including Türkiye. In this country, MoAF has been engaged in efforts to control and eradicate this disease for many years. However, despite these efforts, the desired success has not yet been achieved, due to various reasons. Mass vaccination of bovine against brucellosis was identified as the most cost-effective measure compared to alternative methods. Therefore, it is recommended to utilize a vaccine strain that ensures robust immunity while also maintaining safety for both public and animal health.

In 2011, the burden of infection on bovine populations was reduced through the conditional slaughter and culling of bovine reservoirs of *B. abortus*, along with the disinfection of premises where the disease was present. As part of the project, Çankırı province was among the first provinces to implement a mass conjunctival vaccination program for bovines in 2012. By 2012 and 2013, a vaccination rate exceeding 82.5% resulted in robust herd immunity. Stakeholders were informed about these initiatives, and their support was garnered. As a result of these concerted efforts, the number of bovine brucellosis outbreaks and the subsequent compensation for affected animals decreased significantly in the subsequent years. While the percentage of outbreaks in Çankırı province, Türkiye, was 11.73% in 2011, this rate decreased to 2.85% in 2015 following the intervention efforts (Table). In addition, as shown in Figure 1, when compared to the 4.5-year total, the number of compensation payments (82.3%) and the amount of compensation paid (86%) were notably high in 2011. These rates were observed to decrease gradually each year in 2012, 2013, 2014, and 2015.1. As a result of this effective fight, in addition to reducing the risk of public and animal health, a significant amount of public resources have been saved and the socioeconomic loss of the sectors has been prevented. Verbal interviews with health institutions in the province indicated a decrease in the number of human cases as well.

After the vaccination of bovines, the cellular immune response is stimulated. The *B. abortus* S19

strain has been effectively used in the field to prevent infection for over eighty years (Dorneles et al., 2014; Dorneles et al., 2015). The availability of both conjunctival and subcutaneous forms of this vaccine, produced by both state and private sectors in Türkiye, offers significant advantages in terms of cost-effectiveness and logistical planning. It's essential to consider the long-term protection and cross-protection against *B. melitensis*. However, several challenges exist regarding the *B. abortus* RB51 strain. The exact nature of its mutations remains unknown, and its high cost, due to the absence of local production, presents obstacles for diagnosis and treatment. Additionally, its resistance to rifampicin and inability to be detected by routinely used serological tests further complicate matters, particularly in human cases (Schurig et al., 2002; Dorneles et al., 2015; Khurana et al., 2021). However, both vaccines can be administered to pregnant animals, although there is a risk of inducing abortion (de Oliveira et al., 2022).

WOAH recommends the use of $5-8 \times 10^{10}$ CFU dose of *B. abortus* S19 strain in female calves aged 3-6 months, along with one or two doses of 5×10^9 CFU administered via the conjunctival route in heifers and cows of all ages (de Oliveira et al., 2022). This recommendation suggests that the project has selected an optimal dose for infection prevention. The success of this research further confirms the validity of this dosage selection.

The number of diseases free premises and cattle must be sufficient to support this endeavour. Furthermore, vaccination of cattle in the vicinity of disease-free premises can significantly reduce the pathogen load, thereby minimizing the risk of disease transmission to these premises.

Brucellosis stands as a significant public health concern globally. Hence, it is imperative to foster collaborative efforts with all stakeholders within the framework of the One Health approach to reduce the prevalence of this disease. The MoAF and the Ministry of Health should both allocate funding to combat this ailment effectively. Raising awareness among relevant stakeholders, fostering a shared understanding, and maintaining open channels of communication are paramount for success in this endeavour (Bartels et al., 2012; Khurana et al., 2021). Disseminating information to breeders and their associations about the disease and vaccination through brochures has proven effective in increasing vaccination rates. Additionally, adopting practices such as burying discarded or deceased offspring and fetal membranes due to infection, rather than disposing of them into the environment, helps to reduce potential transmission sources. Moreover, individuals including breeders, caretakers, and workers who are educated about the zoonotic nature of the disease demonstrate increased vigilance against self-infection.

CONCLUSION

As demonstrated by the example of Çankırı province, it is evident that controlling bovine brucellosis in Türkiye is feasible, albeit requiring substantial financing and a long-term commitment. Given the high prevalence of the disease in bovine herds, it is deemed achievable to attain eradication by initially reducing the prevalence below 1% through vaccination. Subsequently, a regional eradication strategy can be implemented gradually, utilizing the test-and-slaughter method. Efficient planning and uninterrupted provision of financing and free breeding stock are imperative at this stage. Adequate resources should be allocated to meet the breeding cattle needs of areas where the disease has been eradicated.

Activities should be meticulously planned and carried out in coordination as a state policy throughout the country. During the planning phase, various modelling approaches should be explored, prioritizing the selection of the most cost-effective option. When implementing test-and-slaughter

policies, public health considerations should take precedence, and the Meat and Milk Institution should be tasked with the public responsibility of slaughtering conditional animals. Given the advantages and risks, both subcutaneous and conjunctival formulations of the *B. abortus* S19 strain are recommended for use in Türkiye. To achieve optimal herd immunity, vaccination campaigns should aim for a coverage rate of 80% or higher. Effective communication with stakeholders and raising their awareness are vital for achieving set targets. Proper identification of animals, accurate record-keeping, and monitoring of their movements are essential components of successful disease management.

MoAF should promptly implement specialty training programs in Veterinary Medicine, while simultaneously increasing the number of specialized personnel in epidemiology and related fields, along with enhancing their professional rights. Support for research on vaccine development and disease diagnosis should be prioritized, with efforts directed towards updating diagnostic methods to align with international standards. Encouraging the training of specialized human resources is essential. Additionally, there should be an emphasis on expanding research and development capacity to create new vaccines and adjuvants that are safer, more effective, and suitable for field conditions.

In conclusion, since bovine brucellosis is a zoonosis and all stages of the fight against the disease require field expertise, a “One Health” approach should be adopted, and multidisciplinary studies should be carried out. It should not be forgotten that the protection of public health depends on the health of animals and food.

Acknowledgements

The authors thank everyone who contributed to this fight for their support.

Ethical Statement

Since retrospective data were used in this study, there was no need to obtain ethical permission.

Author Contributions

Research Design (CRediT 1) Author 1 (%60) – Author 2 (%40)

Data Collection (CRediT 2) Author 1 (%80) – Author 2 (%20)

Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%50) – Author 2 (%50)

Writing the Article (CRediT 12-13) Author 1 (%60) – Author 2 (%40)

Revision and Improvement of the Text (CRediT 14) Author 1 (%50) – Author 2 (%50)

Funding

There is not any funding in this study.

Conflict of Interest

Authors declare that there is no conflict of interest.

Sustainable Development Goals (SDG)

2 Zero hunger

3 Good health and well-being

8 Sustainable economic growth

12 Ensure sustainable consumption and production patterns

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Effect of Age at First Calving and Season on First Lactation Milk Yield, Lactation Period and Calves Growth Data in Anatolian Water Buffaloes in Çorum Region

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Article Info

ABSTRACT

Article History

Received: 24.05.2024

Accepted: 11.10.2024

Online First: 04.12.2024

Published: 23.01.2025

Keywords:

Anatolian water buffalo,
First calving,
Lactation milk yield,
Lactation period,
Correlation.

This study was carried out using the data of the National Anatolian Water Buffaloes (AWB) Breeding in Farm Project, which is being carried out in Çorum Region. Data of 292 AWB gave birth between 2019 and 2023 and their calves (n=292) raised in 30 enterprises were used. Age at First Calving (AFC), Lactation Milk Yield (LMY), Lactation Period (LP), Daily Average Milk Yield (DAMY), Calf Birth Weight (CBW), Calf Gender (CG), and Calving Season (CS) were evaluated. The highest milk yield was detected in the buffaloes that were first bred after 50 months while the lowest LMY was observed in buffaloes with AFC less than 38 months ($p=0.003$). According to data, an increase in milk yield can be expected as AWB get older. Furthermore, the difference between LP and AFC and calving season, sex of calves were not significant. The difference between DAMY and the effect of AFC and CS was statistically significant ($p<0.05$). While the LMY of the buffaloes with the highest AFC were high, there was a difference between the other two groups. The milk yield of the AFC in summer was significantly lower than in other seasons ($p<0.05$). AWB with high AFC had high LMY and prolonged lactation period. When AFC was in the summer months, LMY and LP decreased, so arranging calving in the spring can be recommended. In addition, although the effect of first breeding at older ages appears to be positive on CBW, the disadvantage of first breeding at older ages in terms of sustainable herd management should also be considered.

To cite this article:

Yılmaz, M.A., Sevgi, R., Ünay, E., Coşkun, M.İ., Okuroğlu, A., Yıldırım, M., Bülbül, B., Sarıkaya, Ö., Kaplan, Y., & Özdemir, A. (2025) Effect of Age at First Calving and Season on First Lactation Milk Yield, Lactation Period and Calves Growth Data in Anatolian Water Buffaloes in Çorum Region. *Research and Practice in Veterinary and Animal Science (REPVAS)*, 2(1), 22-33.

<https://doi.org/10.69990/REPVAS.2025.2.1.3>

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INTRODUCTION

All foods of animal origin play a major importance in human nutrition (Say et al., 2021, Şenyüz et al., 2022, Uysal et al., 2024). They contain proteins, vitamins, and minerals necessary for human (Şenyüz et al., 2022; Oyan et al., 2024; Yılmaz et al., 2024). As the world population, people's need for animal protein also increasing (Yıldırım et al., 2011; Sevgi et al., 2019; Yılmaz et al., 2024). In order to meet this demand, preferences, and strategies are changing in the animal food production sector (Chaudhari, 2015; Yılmaz et al., 2024). With changing preferences, new strategies need to be developed (Moioli, 2005; Say et al., 2016). One of these strategies is the effective use of genetic supply from the past (Yıldırım et al., 2011; Satılmış et al., 2021; Şenyüz et al., 2022). These include the breeding of local cattle, small ruminant and buffalo breeds (Yıldırım et al., 2011; Say et al., 2021; Satılmış et al., 2021; Şenyüz et al., 2022; Yılmaz et al., 2024). Buffaloes are seen as the livestock of the bride in most countries due to many factors such as their resistance to most diseases, ability to utilize roughage well, and quality milk properties (Borghese, 2010; Uğurlu, 2017; Yılmaz et al., 2024). In addition, it is estimated that buffaloes will have an easy adaptation ability to the difficult conditions that will occur in a possible climate change (Nanda and Nakao, 2003; Soysal et al., 2005; Borghese, 2010; Uğurlu, 2017; Yılmaz et al., 2024). Buffaloes have a great economic value, especially in various regions of Asia (India, China, Pakistan, Indonesia, and the Philippines) as a farm animal, and they are bred for their meat, milk, and power (Moioli, 2005). Approximately 95% of the 200 million buffaloes in the world are in Asia and this is constantly increasing (FAO, 2020). It is known that water buffaloes in Anatolia date back to 3000 BC (Yılmaz et al., 2024). However, it turned out that domesticated water buffaloes were brought to Anatolia much later. The buffaloes found in the region are genetically close to the Mediterranean buffalo (Italian buffalo), a subgroup of the river buffalo. Today, there is a specific breed called AWB in Anatolia. These buffaloes are considered to be a separate or smaller subgroup of Mediterranean buffaloes (Şahin and Küçükkebaççı, 1999; Soysal, 2013; Uğurlu, 2017; Yılmaz and Kara, 2019).

Buffaloes can efficiently utilize roughages and by-products of many agricultural crops. They are also highly resistant to diseases (Nanda and Nakao, 2003; Yılmaz et al., 2024). In both cattle (Eastham et al., 2015) and buffaloes (Hossein-Zadeh, 2016; Sathwara et al., 2020), the first lactation yield traits are interrelated as they affect subsequent lactation performance. Age at First Calving (AFC) is the major reproductive trait that influences herd productivity and profitability due to its impact on both breeding cost and future yield performance of the animal (Soliman and Sadek 2004; Krpalkova et al., 2014; Eastham et al., 2018). Buffaloes have been accepted as the best breeding animal for poor farmers from past to present due to their unique animal products (meat, milk, cream, etc.), utilization of their power, and high utilization of poor-quality roughages. Feeding and administrative costs are low in buffaloes compared to cattle (Şahin and Küçükkebaççı, 1999; Atasever and Erdem, 2008; Chaudhari, 2015; FAO, 2020; Yılmaz et al., 2024).

Various factors are effective on Lactation Milk Yield (LMY) and Lactation Period (LP) in buffaloes (Cady et al., 1983; Khan and Chaudhry, 2000; Afzal et al., 2007; Patbandha et al., 2015; Verma et al., 2018). One of these is the AFC and it is usually expressed in months (Alim and Ahmed, 1954; Chaudhry and Ahmed, 1978; Sreedharan and Nagarcenkar, 1987). Early AFC has positive contributions to fertility in both cattle (Krpalkova et al., 2014; Mokhtari et al., 2015) and buffaloes (Thiruvankadan et al., 2015). In addition, it is known that AFC has a relationship with reproductive parameters and yield values throughout the life of the individual (Khan and Chaudhry, 2000; Afzal et al., 2007; Tamboli et al., 2022). There are also studies reporting that the potential benefits of earlier calving in buffaloes but these are few (Ali et al., 1999; Borghese et al., 2005). In buffaloes with low AFC, the profitability of the auxiliary dairy enterprise increased by reducing the cost of production (Lopez-Paredes et al., 2018). However, as in domestic animals, it is necessary to understand the production and reproductive potentials as well as the relationships between them to determine strategies in breeding programs in

buffaloes (Malhado et al., 2013; Hossein-Zadeh, 2016). Many studies have been conducted to estimate the heritability, genetic and phenotypic correlations of yield traits in buffalo breeding (Catillo et al., 2002; Breda et al., 2010; Hossein-Zadeh, 2016; Sathwara et al., 2020; Tamboli et al., 2022).

This study was carried out to analyze the effect and relationship of various factors on milk production in AWB reared semi-extensively in farms located in the north of Anatolia. For this purpose, correlations and interactions between LMY, LP, DAMY, AFC, Calf Birth Weight (CBW), Calf Gender (CG), and Calving Season (CS) data were evaluated. With the help of these data, it was tried to determine the ideal AFC and birth season for higher milk yield in the specified region.

MATERIAL and METHOD

Study Material

This study was conducted within the scope of the sub-project of the National Anatolian Water Buffaloes Breeding in Farm Project carried out in Çorum Region. They were fed *ad-libitum* in farms. The lactating all buffaloes grazed outside between the months of late March to early December, while being kept and fed indoors through the winter.

Method and Data Collection

In this study, data of 292 AWB gave birth for the first time between 2019 and 2023 and their calves (n=292) raised in 30 farms were used. All data were collected by the same technical personnel and recorded in the Manda Yıldızı program (Tekerli, 2022) as soon as they were received. LMY, LP, Daily Average Milk Yield (DAMY), AFC, CBW, CG, and CS data were used in the study. In the farms where the project was carried out, the weights of the calves born were taken on the first day and the milk yields of the buffaloes were taken monthly. The interpolation method was used to calculate lactation milk yields; ie, data from at least 4 or 5 months of milk control during the lactation period were used (Tekerli, 2022).

Statistical Analysis

Data of LMY, LP, and DMY were analyzed using the general linear model. The fixed effects considered the first age of birth in three classes (25-37 months, 38-49 months, and 50+ months), gender of the calf in 2 classes (male and female), and the season of birth in 4 classes (spring, summer, autumn, and winter). All analyses were performed by R statistical software. The differences between the means of the sub-groups were tested by the Tukey test (Petrie and Watson, 2013; Selvi, 2024) ($p < 0.05$).

RESULTS

In the study, the first calving age were divided into three groups as 25-37 months, 38-49 months, and 50+ months and the number of sire buffaloes in AFC groups were 99, 94, and 99, respectively. The average LMY was 900.8 kg, 986.9 kg, and 1010.3 kg, and LP was 226 days, 233 days, and 227 days in AFC groups, respectively. In terms of general averages, means of LMY, LP, and DAMY were 956.0 ± 17.41 kg, 228.5 ± 2.58 days, and 4.2 ± 0.07 kg, respectively (Table 1). DAMY was 3.9 kg, 4.3 kg, and 4.5 kg in AFC groups, respectively (Table 1). According to the age at first calving of the buffaloes, the highest milk yield was in buffaloes calving for the first time after 50 months ($p = 0.003$). The lowest LMY was in buffaloes whose age at first calving was less than 37 months. An increase in milk yield can be expected with increasing age. However, although the milk yield of the buffaloes that were first milked between the ages of 38-49 months was not statistically different, it was 86 kg higher than the buffaloes

that were not first milked. The effects of AFC and season were significant ($p < 0.05$). While DAMY was the highest in the buffaloes with the highest AFC there was no difference between the other two groups. The milk yield of buffaloes mulched in summer was significantly lower than in the other seasons. According to the season of calving (CS), the number of first calving buffaloes was 164 (56%), 78 (27%), 14 (5%), and 36 (12%) in spring, summer, autumn, and winter seasons, respectively. According to the seasons, LMY was 968.7 kg, 903.5 kg, 993.2 kg, and 998.5 kg, and LP average was 224 days, 224 days, 232 days, and 234 days, respectively. DAMY was 4.3 kg, 4.1 kg, 4.3 kg, and 4.3 kg according to the seasons, respectively (Table 1).

The number of male and female calves born were 134 and 158, respectively. The average, LMY was 954.0 kg and 977.9 kg in male and female calving in AWB, respectively, and the average LP was 228.5 days and 228.5 days, respectively. CBW according to the calves gender was close to each other (4.2 kg and 4.3 kg, respectively). There was no significant difference between the calf genders in terms of LMY and LP (Table 1).

Table 1. Means of lactation characteristics of Anatolian Water Buffalo (\pm SEM.).

Factor	n	Lactation milk yield (kg)	Lactation period (d)	Daily milk yield (kg)
Age (month)				
25-37	99	900.8 \pm 40.39 ^b	225.9 \pm 5.98	3.9 \pm 0.17 ^b
38-49	94	986.9 \pm 21.54 ^b	232.6 \pm 3.19	4.3 \pm 0.09 ^b
50+	99	1010.3 \pm 21.07 ^a	227.0 \pm 3.12	4.5 \pm 0.09 ^a
p		0.003	0.183	0.004
Calving Season				
Spring	164	968.7 \pm 11.46 ^a	223.8 \pm 1.70	4.3 \pm 0.05 ^a
Summer	78	903.5 \pm 17.68 ^b	223.9 \pm 2.62	4.1 \pm 0.08 ^b
Autumn	14	993.2 \pm 60.77 ^a	232.4 \pm 9.00	4.3 \pm 0.26 ^{ab}
Winter	36	998.5 \pm 26.32 ^a	233.8 \pm 3.90	4.3 \pm 0.11 ^{ab}
p		0.010	0.315	0.029
Gender				
Male	134	954.0 \pm 25.24	228.5 \pm 3.74	4.2 \pm 0.11
Female	158	977.9 \pm 18.42	228.5 \pm 2.73	4.3 \pm 0.08
p		0.138	0.522	0.199
General	292	956.0\pm17.41	228.5\pm2.58	4.2\pm0.07

^{a, b}: Means of the same parameter in the same column with different superscripts differ significantly ($P < 0.05$).

Calves' birth weights averaged as 27.4, 28.7, and 30.2 kg in AFC groups, respectively (Table 2); The calves' birth weights were 29.7, 29.3, 26.8, and 29.2 kg according to season, respectively; The number of male and female calves were 134 and 158, respectively, and their birth weights averaged 28.9 and 28.5 kg, respectively (Table 2). Although there was no statistical difference according to season and gender of the calves, there was a tendency being significant for the differences according to age at birth ($p = 0.087$) (Table 2).

Phenotypic correlations and interactions between the investigated traits showed that there were positive correlations between AFC and CS, MA, LMY, LP, and DAMY, between CS and LP, between CBW and LMY, LP and DAMY, between LMY and LP and DAMY, negative correlations between DM and CBW, LMY and DAMY, and between LP and DAMY (Table 3).

Phenotypic correlations and interactions between AFC, BM, BW, LMY, LP and DAMY traits were calculated in statistical analyses (Table 3).

Table 2. Means of birth weights of Anatolian Water Buffalo calves according to age at calving (month), season and calf gender (\pm SEM.).

Factor	n	Birth weight (kg)
Age (month)		
25-37	99	27.4 \pm 1.13
38-49	94	28.7 \pm 0.61
50+	99	30.2 \pm 0.59
<i>p</i>		0.087
Calving Season		
Spring	164	29.7 \pm 0.32
Summer	78	29.3 \pm 0.49
Autumn	14	26.8 \pm 1.70
Winter	36	29.2 \pm 0.74
<i>p</i>		0.644
Gender		
Male	134	28.9 \pm 0.71
Female	158	28.5 \pm 0.52
<i>p</i>		0.272
General	292	27.7\pm0.49

Table 3. Phenotypic correlations among traits.

	BM	BW	LMY	LP	DAMY
AFC	0.127*	0.217**	0.233**	0.085	0.189**
BM		- 0.024	- 0.088	0.013	- 0.094
BW			0.202**	0.072	0.156**
LMY				0.412**	0.781**
LP					- 0.234**

* Correlation is significant at $P < 0.05$. ** Correlation is significant at $P < 0.01$. AFC; age at first calving, BM; birth month, BW; birth weight, LMY; lactation milk yield, LP; lactation period, DAMY; daily average milk yield.

DISCUSSION

AFC was reported as 37.64 ± 3.19 months in Italian Buffaloes that live in Anatolia (Özbaşer et al., 2022), 37.4- 39.4 months in Egyptian Buffaloes (Alim and Ahmed, 1954), 47.06 months in Nili Ravi Buffaloes (Chaudhry and Ahmed, 1978) and 41 months in Murrah Buffaloes (Sreedharan and Nagarckenkar, 1987; Tamboli et al., 2022). The effect of AFC on LMY, LP, and CBW has been reported by many researchers (Alim and Ahmed, 1954; Sreedharan and Nagarckenkar, 1987; Chaudhary and Ahmed, 1978; Özbaşer et al., 2022).

In livestock, especially in extensive systems with high dependence on nature, it is expected that the birth season will be intensified in the spring season when feed resources are high. In the semi-extensive AWB breeding system in Çorum conditions, it was observed that the births were

concentrated in spring. On the other hand, it was determined that the buffaloes AFC in the spring period, which had the highest birth rate, had approximately 65 kg more LMY compared to the buffaloes AFC in the summer period ($P = 0.010$). Afzal et al. (2007) found significant effect of the first calving season on LMY and LP. They also reported that milk yield was higher in spring than in summer in the first calving season (Aflaz et al., 2007).

In the present study, the highest LMY in terms of AFC was observed in buffaloes that were AFC of 50+ months ($p = 0.003$). The lowest LMY was observed in buffaloes whose AFC was less than 37 months. An increase in milk yield can be expected with increasing age. The effects of AFC and season were found to be statistically significant ($p < 0.05$). While DAMY was found to be the highest in the buffaloes with the highest AFC there was no difference between the other two groups. The milk yield of the buffaloes that were calving in summer was found to be significantly lower than in the other seasons. These data are close to some previous studies in Indian local buffaloes (Verma et al., 2018), Italian buffaloes (Catillo et al., 2002), Nili Ravi buffaloes (Cady et al., 1983; Chaudhry, 1992; Khan and Chaudhry, 2000; Afzal et al., 2007), in Murrah buffaloes (Pandey et al., 2015; Tamboli et al., 2022) and Jaffrabadi buffaloes (Patbandha et al., 2015). It has been reported by many researchers that breeding, management and nutrition conditions in different regions have also affect on these parameters (Cady et al., 1983; Khan and Chaudhry, 2000; Afzal et al., 2007, Pandey et al., 2015; Patbandha et al., 2015; Verma et al., 2018).

In the present study, the effects of AFC, season, and CG on LP were not significant while the effects of AFC and season on DAMY were significant ($p < 0.05$). The buffaloes with the highest age at first calving ($50 \leq$ months) had the highest DAMY, but there was no statistical difference between the other two groups. Verma et al. (2018) classified the buffaloes as two groups with an average calving age of 36 or 48 months, and determined the LP as 309.35 ± 15.13 and 344.80 ± 13.36 days in the first lactation period, respectively. The AWB data were close to the data of Verma et al. (2018) in Indian buffaloes and Afzal et al. (2007), Cady et al. (1983) and Khan and Chaudhry (2000) in Nili Ravi buffaloes. In addition, the data of our study are lower than that obtained by the studies conducted on Nili Ravi buffaloes (Chaudhry, 1992), Murrah buffaloes (Pandey et al., 2015), Italian buffaloes (Catillo et al., 2002) and Jaffrabadi buffaloes (Patbandha et al., 2015). Along with the regional, maintenance and feeding differences, breed is also a major factor which is responsible for the differences of the data among the studies (Cady et al., 1983; Chaudhry, 1992; Afzal et al., 2007; Krpalkova et al., 2014; Verma et al., 2018, Tamboli et al., 2022).

In the present study, phenotypic correlations and interactions were evaluated between AFC, BM, BW, MY, LP and DAMY. Verma et al. (2018) reported that high AFC had a weak correlation between (- 0.374 and 0.238) and (0.024 and -0.133) interactions for lactation length. The phenotypic correlation data of this study were close to the data of Verma et al. (2018) in Indian buffaloes and Afzal et al. (2007) in Nili Ravi buffaloes. Afzal et al. (2007) reported a high correlation between CS, LMY and LP. In addition, the same researchers found a high correlation between both LMY and LP in buffaloes calved first in spring and in summer. Although the interaction and correlation data of this study were similar with the results of Afzal et al., 2007 (Indian buffaloes), Cady et al., (1983) and Khan and Chaudhry (2000) (Nili Ravi buffaloes), there were difference with the studies conducted by Catillo et al., (2002) (Italian buffaloes), Chaudhry (1992) (Nili Ravi buffalo), Pandey et al., (2015), Tamboli et al., (2022) (Murrah buffaloes) and Patbandha et al., (2015) (Jaffrabadi buffaloes). It has been reported by many researchers that these correlations may be due to genetic parameters, maintenance and feeding conditions (Cady et al., 1983; Khan and Chaudhry, 2000; Catillo et al., 2002; Afzal et al., 2007; Krpalkova et al., 2014; Pandey et al., 2015; Verma et al., 2018; Patbandha et al., 2015; Tamboli et al., 2022).

CONCLUSION

AWB with high AFC have high daily milk yield and their lactation period is increased. LMY of the buffaloes which first calved in summer is lower than those in other seasons. Therefore, it may be recommended to calve in spring. In the present study, positive correlations between AFC and CS, CBW, LMY, LP, DAMY; between CS and LP; between CBW and LMY, LP, and DAMY, and negative correlations between LMY and LP, DAMY; between DM and CBW, LMY, DAMY; between LP and DAMY were found. The characteristics showed that AFC between 38-49 months was ideal in AWB and CS in spring was effective on milk yield and LP. In addition, although the effect of AFC 50+ months of age on CBW seems to be positive, the disadvantage of AFC at older ages in terms of sustainable herd management should also be considered.

Acknowledgements

The authors are thankful to the Ministry of Agriculture and Forestry, General Directorate of Agricultural Researches and Policies, which is the coordinator of the Anatolian Water Buffalo Breeding in Farms Project; International Center for Livestock Research and Training technical personnels who is working in project; Çorum Provincial Directorate of Agriculture and Forestry, which carried out the official controls of the project; and Çorum Province Water Buffalo Breeders Association, which provided the logistically their contributions to this study.

Ethic Approval

Ethics Committee permission was granted to the study with the decision of International Livestock Research and Training Centre, Animal Experiments Local Ethics Committee (approval no: 26.02.2021/187).

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Writing the Article (CRediT 12-13) Author 1 (%50) – Author 2 (%10)- Author 3 (%10)- Author 4 (%10)- Author 8 (%10)- Author 9 (%10).

Revision and Improvement of the Text (CRediT 14) Author 1 (%50) – Author 2 (%10)- Author 6 (%10)- Author 7 (%10)- Author 8 (%10)- Author 9 (%10)

Funding

This study was conducted within the scope of the sub-project of the National Anatolian Water Buffaloes Breeding in Farm Project in Çorum Province. Project Number: TAGEM/19MANDA/2012-01.

Conflict of Interest

The authors declare that they have no conflict of interest.

Sustainable Development Goals (SDG)

2 Zero Hunger

3 Good Health and Well-Being

12 Responsible Consumption and Production

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Evaluation of Fertility Parameters and Nutrition-Fertility Relationship in Dairy Cattle Farms in Ereğli District of Konya Province

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Article Info

ABSTRACT

Article History

Received: 25.05.2024

Accepted: 16.10.2024

Online First: 04.12.2024

Published: 23.01.2025

Keywords:

Animal nutrition,
Dairy cattle breeding,
Reproductive parameters

In this study, 49 dairy farms (total cow number: 2234) in Ereğli district of Konya province were surveyed using a face-to-face questionnaire to obtain information about the dairy farms animal nutrition, care, and reproductive efficiency of dairy farms. The research material consisted of the data obtained from breeders through the questionnaire. The mean values of the calving interval and number of days open were 374.73 (days), and 95.61 (days), and the number of artificial inseminations and natural mating per pregnancy were 2.27 and 1.7 respectively. In our study, the mean duration of the first postpartum estrus was found to be 44.30 days and 71.42% of the dairy farms had estrous between 20-45 days. It was observed that 95.9% of the dairy cattle owners thought that the energy level of the ration, 85.7% of the protein level of the ration, and 73.5% of the vitamin-mineral supplementation were effective on fertility. It was observed that only 10.2% of dairy cattle owners used pasture for nutritional purposes and 89.8% of those who used pasture thought that pasture was inadequate. It is also observed that the breeders in the region are aware of the nutritional conditions. It is also observed that the dairy farmers in the area are aware of the nutritional conditions. In conclusion, it is observed that the dairy cattle farms in the Ereğli district of Konya province are not effective in terms of the reproductive parameters evaluated in the study. Also, these results regarding grazing clearly show that grazing is insufficient in terms of animal nutrition.

To cite this article:

Sarı, A., Ayaş, R. & Selvi M.H. (2025). Evaluation of Fertility Parameters and Nutrition-Fertility Relationship in Dairy Cattle Farms in Ereğli District of Konya Province. *Research and Practice in Veterinary and Animal Science (REPVAS)*, 2 (1), 34-48.

<https://doi.org/10.69990/REPVAS.2025.2.1.4>

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INTRODUCTION

The profitability of dairy cattle farms is directly related to milk yields. Milk yield levels are affected by many cow-related factors such as the genetic structure of the animal, calving age, dry period, service period (days open), or calving interval (Akbulut and Haussmann, 2011). Many of these factors are directly related to reproductive efficiency and fertility parameters. In order to maintain profitability in dairy farms, it is necessary to have one calf per year from a cow (Pekçok and Aksu, 2015; Xu et al., 2000). Therefore, the profitability of dairy cattle farms can only be improved by optimizing fertility parameters (Yılmaz and Sarıözkan, 2020).

The importance of reproductive parameters, which include reproductive characteristics as well as yield characteristics of animals, should not be ignored in order to gain economically in an enterprise. Within physiological and economic limits, it is necessary to maintain reproductive parameters on herd basis in appropriate periods and at appropriate rates. Among these parameters, age at first insemination and first calving, service period, calving interval, first postpartum estrus interval and estrus time determination rate, conception interval and rate at first postpartum insemination, artificial insemination index, total pregnancy rate are generally taken into consideration (Sönmez, 2012).

The age range of heifers suitable for mating or insemination is associated with body weight. It is reported that the age of first calving should be at the age with the most appropriate body weight in order to reduce feed costs in the breeding of heifers and to avoid loss of fertile period time (Akins, 2016). The increase in body weight may vary depending on genetics, breed characteristics or whether the heifers are individually large or small (Hoffman, 2007). For this reason, Van Amburgh and Meyer (2005) suggested evaluating the age at first insemination or first calving based on the heifers having gained a certain percentage of their mature body weight. It is stated that heifers should have gained 55% and 94% of their body weight for first insemination and first calving age, respectively, in order to maximize milk yield (Van Amburgh and Meyer, 2005).

The service period is defined as the period from the birth of the animal until the animal becomes pregnant again. It has been reported that the most appropriate service period in terms of fertility parameters should not exceed 60-90 days. In addition, this period was reported to be 120 days for cows with high milk yield (Ata, 2013; Sönmez, 2012).

The calving interval is defined as the time between two births. The ideal value is reported to be 365 days, but this interval is often reported to be as long as 390 days. The first estrus after calving should be detectable within the first 40 days after calving, while the first insemination or natural mating time after calving should be on the 60th day in healthy cows (Ata, 2013). In order to ensure fertility parameters after calving, it is reported that the estrus detection rate should be at least 80% in the first 60 days and the conception rate should be at least 60% in the first insemination after calving (Sönmez, 2012).

The number of artificial inseminations/natural mating required for each pregnancy to occur in the herd is defined as the artificial insemination index (Kaya et al., 1998). In natural mating, 1.2-1.3 mating for each pregnancy is considered normal in terms of fertility parameters. However, in the case of artificial insemination, this ratio should not be higher than 1.65 inseminations for each pregnancy (Akins, 2016). The pregnancy rate can be calculated separately for each insemination or it can be calculated as the total pregnancy rate with the sum of all inseminations performed in that year. It is calculated as the ratio of the total number of inseminations performed in a given period to the total

number of cows and/or heifers conceived after insemination. The total pregnancy rate should be at least 50% (Dinç and Kutlu, 2015; Parkinson and Barrett, 2009).

Nutrition is one of the factors that affect the proper range of fertility parameters. There is an important relationship between nutrition and fertility. If ruminants are not fed a balanced and adequate diet, fertility may be impaired. Basically, energy level, protein level, fat level, vitamin-mineral level, mycotoxins and anti-nutritional factors in the ration affect fertility.

The energy level of the ration is one of the most important factors affecting fertility. If there is not enough energy in the ration to meet the animal's life and yield requirements, a negative energy balance (NEB) is formed. It has been reported that postpartum uterine involution is delayed, the reconception period is prolonged, the number of inseminations per pregnancy increases, oocyte quality decreases, and infertility may occur in cattle with NEB (Spicer et al., 1990; Yoshida et al., 2007). If the energy level of the ration is higher than the requirement, cows will develop adiposity. As a result of obesity, the body condition score of cows increases. It has been reported that animals with excessive body condition scores, especially in the dry period, rapidly weaken after parturition and their gestation periods are prolonged. (Michael et al., 2019).

If the ration protein level is insufficient, there may be irregularities in the estrous cycle in cattle (Yolcu, 2024). In addition, protein deficiency affects reproductive hormones such as IGF1, estradiol 17 β and progesterone and may cause negative effects on ovarian functions (Hayati et al., 2021). In studies, it was reported that the number of inseminations per pregnancy and the service period increased when the ration protein level was high (Rhoads et al., 2006; Sonderman and Larson, 1989). In addition, barley, which is an important feedstuff in closing the shortage of concentrate feed (Oral and Veziroğlu, 2023), can cause acidosis as a result of incorrect ratios used in nutrition, and this situation can also negatively affect fertility.

Deficiency or excess of minerals and vitamins also affect fertility. If calcium deficiency is taken into consideration, it causes a decrease in uterine muscle contractions after birth, delays uterine involution and negatively affects fertility. It has been reported that manganese deficiency causes an irregular estrous cycle, suboestrus, an increase in ovarian cysts and a decrease in fertility (Yolcu, 2024).

The storage of feedstuffs used for nutritional purposes under the right conditions is also very important for the quality of the feedstuffs and therefore the correct nutrition. During transportation and storage of feed, physical factors such as humidity, temperature, or chemical factors such as carbon dioxide and oxygen can cause mold to form and release mycotoxins (Bryden, 2012; Frisvad, 1995). Aflatoxin, fumonisin, ochratoxin, trichothecene, and zearalenone are examples of mycotoxins found in feed (Liu and Applegate, 2020). Maize is used as an important feed source due to its high yield and adaptability (Yerlikaya and Soyulu). Mycotoxins such as zearalenone can also be formed in corn silage before or after harvest (during silage making) after some fungal contamination (Richard et al., 2007). It has been reported that these mycotoxins can adversely affect fertility by directly affecting the reproductive system or indirectly affecting other organs and systems (Diekman and Green, 1992).

Some nanoparticles and heavy metals accumulated in water ponds or agricultural lands directly or indirectly affect fertility (Sarı et al., 2023; Verma et al., 2018). The contamination of water resources with heavy metals and especially the use of contaminated water in animal husbandry is a critical environmental problem that negatively affects animal health (Özyiğit, 2021). In addition to nanoparticles and heavy metals in water and feed used in animal nutrition, there may be excessive amounts of nitrate in some cases (Ayaş, 2024). While excessive nitrate intake by ruminants causes

sudden death, low nitrate intake negatively affects both milk yield and fertility (Davison et al., 1964). Fertility problems can also occur in pasture-fed animals. The main reason for this situation is the deterioration of existing pastures due to overgrazing and lack of necessary breeding work over time, and the inability of the animals to meet the nutrient requirements necessary for reproduction and productivity (Armağan and Işık, 2022).

This study aimed to investigate dairy cattle farms in Ereğli district of Konya province in terms of some fertility parameters and nutritional conditions and to evaluate the relationship between fertility and nutrition.

MATERIALS and METHODS

This study was carried out by face-to-face interviews with the 49 owners of dairy cattle farms in Ereğli district of Konya province for a period of 1-3 months. The main material of the study consists of the answers given to the survey questions. There were a total of 2234 cows in all 49 farms, and a total of 35 bulls in 16 farms within these farms. The breeds in the farms were distributed as Holstein (100%), Simmental (48.97%), Holstein-Simmental cross (26.53%) and other breeds (14.28%), respectively.

The first estrus time after calving and the first insemination/mating age were obtained with the information provided by the breeders as an average. The service period was obtained by asking the breeders about the time from calving to the time the cows became pregnant. The calving interval was obtained by adding the pregnancy period to the time from calving to the time they became pregnant, again according to the information provided by the breeders. The obtained data were obtained as the average of all animals on the farm, not as individual data. The number of artificial inseminations/matings required for each pregnancy in the herd was obtained by asking the breeders how many artificial inseminations/matings a cow had to become pregnant and they were asked to state the average.

In dairy cattle farms in Ereğli district, calving interval, service period, first insemination age and the number of insemination/mating per pregnancy and first postpartum estrus values were considered as basic fertility criteria and the target values of these criteria were accepted as ≤ 390 , ≤ 90 , \geq Body Weight 55%, ≤ 1.65 , ≤ 40 , respectively.

In determining the number of farms, the principle of Yamane and Esin (2006) that at least 3% of the sample volume or 10% of the sample volume as stated by Cochran (1977) is sufficient was taken into consideration. In addition, it is also reported that the sample volume increases its ability to better represent the main population as the number of units increases (Sümbüloğlu and Sümbüloğlu, 2017). Dairy farms were randomly selected. The obtained data were analyzed by frequency analysis in SPSS 21.0 statistical program, and numerical and proportional values were obtained (Düzgüneş et al., 1983; Selvi, 2024).

RESULTS

In line with the answers to the questionnaire questions directed to 49 dairy cattle farms in Ereğli district of Konya province, data such as gender, age, education level, age of the owner, farm structure, total number of cattle, distribution of cattle breeds, number of heifers, number of lactating cows, number of pregnant cows, average daily milk yield were evaluated and presented in Table 1.

Table 1. Data on dairy cattle farms

		N	%
Age of owner	21-45	21	42,85
	46-60	22	44,89
	61-74	6	12,24
Gender of owner	Male	45	91,8
	Female	4	8,2
Education level of owner	Literate	3	6,1
	Primary school	28	57,1
	Highschool	10	20,4
	University	6	12,2
	Postgraduate	2	4,1
Number of workers	1-3	47	95,9
	4-5	2	4,1
Age of farms	1-9	12	24,49
	10-19	14	28,57
	20-29	16	32,65
	30-39	3	6,12
	40-44	4	8,16
Type of farm	Open	6	12,2
	Semi-open	43	87,8
Total cattle number	0-19	19	38,77
	20-30	9	18,36
	31-45	7	14,28
	46-90	9	18,36
	91-300	5	10,20
Number of lactating cows	0-15	24	48,97
	16-35	16	32,65
	36-150	9	18,36
Number of heifers	0-10	31	64,58
	11-22	11	22,91
	23-100	6	12,50
Number of pregnant cows	0-20	36	76,59
	21-54	11	23,40
Breeds found in farms (%)			
	Holstein	49	100
	Simmental	24	48,97
	Crossbreed (HoxSim)	13	26,53
	Other breeds	7	14,28
Proportional distribution of breeds		Cow number	
	Holstein	1589	49
	Simmental	285	24
	Crossbreed	213	13
	Other breeds	138	7
	Total	2234	49
Daily milk average	0-23	26	53,06
	24-28	18	36,73
	29-51	5	10,20
Lactation period	0-280	10	20,40
	281-320	30	61,22
	321-420	9	18,36
Gestation period	270-280	40	81,63
	281-290	9	18,36

N: Number of dairy cattle farms

When Table 1 is analyzed, among the 49 dairy cattle farms, the age of the farm's owner was observed to be between 46-60 years old with a rate of 44.89%, the gender of the farm's owner was male

with a rate of 91.8%, and the educational status of the farm's owners was observed as primary school graduate with a rate of 57.1%.

The age of the enterprise was found to be mostly in the range of 1-18 years with 51.02%. It was observed that out of 49 dairy cattle farms, 43 were semi-open (87.8%) and 6 were open (12.2%). The total number of cattle was determined as 2234. Of the cattle in the dairy cattle farms, 1589 were Holstein (71.12%), 285 were Simmental (12.75%), 213 were Simmental-Holstein cross (9.53%) and 138 were other breeds (6.17%). While the average daily milk of the dairy cattle farms was between 16-23 liters with a rate of 53.06%, it was observed that only 10.20% of the dairy cattle farms had an average daily milk of 29 liters or more. The average lactation period was calculated as 299.37 ± 6.57 and it was determined that 61.22% of the dairy cattle farms lactated for 281-320 days.

In Table 2, data on reproductive parameters such as calving interval, first estrus period after postpartum, days open, age at first insemination, artificial insemination index, as well as the number of cows per bull and which of the conception methods were more preferred were analyzed.

Table 2. Fertility parameters on dairy cattle farms

Fertility Parameters	N	% of farms	
First postpartum estrus	35	71,42	0-45days
	8	16,32	46-60 days
	6	12,24	61-150 days
Calving interval	9	18,36	300-350 days
	33	67,34	351-400 days
	4	8,16	401-450 days
	3	6,12	451-500 days
Days open	32	65,30	0-94 days
	12	24,48	95-134 days
	5	10,20	135-200 days
First insemination age	10-13 months	2	4,5
	14-17 months	29	65,9
	18+ months	13	29,5
First mating age	2	11,8	10-13 months
	12	70,6	14-17 months
	3	17,6	18+ months
No. AI/cow	27	62,79	1-2
	16	37,20	3-5
No. NM/cow	8	50	1-1,9
	8	50	2-3
Breeding method	32	65,3	Artificial Insemination (AI)
	4	8,2	Natural Mating (NM)
	13	26,5	Both of two
No. of cows per bull	16	32,65	7,28 cow

N: Number of dairy cattle farms, NM: Natural mating, AI: Artificial insemination.

It was observed that the calving interval value was between 351-400 days in 67.34% of the dairy cattle farms. When it was requested that the days open should not exceed 90 days, it was determined that it was between 0-94 days in 65.30% of the enterprises. As for the age of first artificial insemination (AI) and first natural mating (NM), it was determined that 65.9% and 70.6% of the owners preferred animals that reached the age of 14-17 months, respectively.

Among the methods used to pregnancy, it was determined that 65.3% of the owners preferred only artificial insemination, 8.2% preferred only natural mating, and 26.5% preferred both artificial insemination and natural mating. When the owners who preferred both methods were asked why they preferred both methods, 66.7% of the dairy cattle farms stated that it was for the guarantee of pregnancy. While 50% of the dairy cattle farms that preferred both methods preferred artificial insemination first, 50% stated that they preferred natural mating first. It was observed that 57.1% of the owners thought that there was a difference between artificial insemination and natural mating in terms of pregnancy success. Of the owners who thought that there was a difference, 65.4% stated that natural mating was more successful, but they reported that they preferred artificial insemination to prevent blood affinity between them. Among the enterprises, only 16 enterprises had bulls. While the average number of bulls per farm was 2.18, the average number of cows per bull was 7.28.

It was found that the animals were removed from the herd after 1-4 lactations in 20.40%, after 5-8 lactations in 46.93% and after 9-11 lactations in 32.65% of the dairy farms. It was observed that 33.3% of the dairy cattle farms preferred to use straw for insemination on the recommendation of their veterinarians and 17.8% of the dairy cattle farms preferred it according to the structure of the animals they breed. It was observed that only 6.8% of the dairy farms used sexed semen. It was observed that the time of first estrus observed after birth was within the desired range of 20-45 days in 71.42% of the dairy farms. The number of natural mating per pregnancy (no. NM/cow) was 1-1.9 matings in 50% of the dairy farms and 2-3 matings in the other 50% of the dairy farms. The number of artificial inseminations per pregnancy (no. AI/cow) was between 1-2 inseminations in 62.79% of the dairy farms. While the mean number of natural mating per pregnancy was 1.70 ± 0.14 , the mean number of AI was 2.27 ± 0.94 . It was clearly observed that both the number of natural matings and the number of artificial inseminations were not among the normal values in terms of fertility parameters. Nevertheless, 73.5% of the owners were satisfied with the pregnancy rates. 67.3% of the owners stated that they did not use hormones. It was found that 50% of those who used hormones did so for treatment purposes, 25% for synchronization purposes and 25% for both synchronization and treatment purposes.

In response to the question "What could be the reason for fertility problems in cows?" presented in Table 3, 28.6% of the breeders stated that the only reason was feeding. In total, 79.4% of the breeders thought that feeding affected reproduction. In our survey, 70.8% of the breeders answered yes to the question: 'Do you think you feed your animals enough?' 43% of the breeders receive technical support in ration preparation. Also, 52.4% of the breeders who receive technical support prefer private consultants. According to our survey, 61.2% of the breeders feed their animals with different rations in different physiological periods. In addition, 95.9% of the breeders believe that the ration energy level affects fertility, and 85.7% believe that the ration protein level affects fertility. While 73.5% of the breeders believe that the ration vitamin and mineral level affects fertility, only 67.3% add vitamin and mineral premix to the ration. 93.9% of the breeders think that feed storage conditions will affect feed quality. Only 10.2% of the breeders use pastures for animal feeding, and 89.8% reported that pastures are insufficient in terms of animal nutrition.

Table 3. Questions and answers about nutrition on dairy cattle farms

Questions and answers about nutrition	N	%
Technical support for ration preparation		
Yes	21	42,9
No	28	57,1
Total	49	100
The same ration for all cattle		
Yes	19	38,8
No	30	61,2
Total	49	100
Effect of ration energy level to reproduction		
Yes	47	95,9
No	2	4,1
Total	49	100
Effect of ration protein level to reproduction		
Yes	42	85,7
No	6	12,2
Total	48	100
Addition of vitamin and mineral to ration		
Yes	33	67,3
No	15	30,6
Total	48	100
Ration change during reproductive period		
Yes	3	6,1
No	46	93,9
Total	49	100
Effect of feed storage conditions to feed quality		
Yes	46	93,9
No	3	6,1
Total	49	100
Effect of mycotoxins to reproduction		
Yes	45	91,8
No	3	6,1
Undecided	1	2
Total	49	100
Sufficient of nutrition		
Yes	34	70,8
No	14	29,2
Total	48	100
Go out to pasture		
Yes	5	10,2
No	44	89,8
Total	49	100
Sufficient of pasture		
Yes	5	10,2
No	44	89,8
Total	49	100
Addition to pasture		
Yes	4	80
No	1	20
Total	5	100

N: Number of dairy cattle farms

DISCUSSIONS

In dairy cattle farms, factors such as the genetic structure of the herd, appropriate herd management, adequacy of care, and nutrition conditions are among the important factors in terms of the economic limits that can be achieved. Therefore, these factors also affect the reproductive parameters of the herd (Inchaisri et al., 2010). Deviation of fertility parameters from target values is reported to lead to financial losses (Sarıözkan et al., 2012).

There is an important relationship between nutrition and fertility in ruminant animals. As a result of our questionnaire study, 28.6% of the farmers stated that the only reason for the fertility problem in cows was nutrition, while 50.8% stated that there were other reasons in addition to nutrition. In total, 79.4% of the farmers think that nutrition affects reproduction.

If ruminants are not fed a balanced and adequate diet, fertility may be disrupted. In the survey we performed, 70.8% of the farmers answered yes to the question 'do you think you feed your animals sufficiently', while 29.2% answered no.

Basically, energy level, protein level, fat level, vitamin-mineral level, mycotoxins and anti-nutritional factors in the ration affect fertility. Therefore, ration preparation is a very important issue and technical support should be obtained. According to our survey, 43% of farmers receive technical support for feed formulation, while 57% do not. Of those who receive technical support, 52.4% prefer private consultants, 23.8% feed dealers, 14.3% veterinarians and 9.5% neighboring dairy farms.

Animals have different nutritional needs at different physiological stages. Different rations should be prepared to meet these needs. Feeding animals with a single ration results in feeding some animals more than necessary and others less than necessary. This situation has a negative effect on fertility. According to our survey, 61.2% of the farmers feed their animals with different rations in different physiological periods, while 38.8% feed their animals with a single ration. Feeding animals with a single ration has advantages in terms of work. However, it can negatively affect both milk yield and fertility.

Ration energy level is one of the most important factors affecting fertility. Excessive ration energy level negatively affects fertility through adiposity. If the ration energy level is lower than necessary, it negatively affects fertility through negative energy balance (NEB) (Michael et al., 2019). According to our survey, 95.9% of the farmers think that ration energy level affects fertility, while 4.1% think that ration energy level does not affect fertility.

The protein content of the diet is another factor affecting fertility. When protein is deficient in the diet, irregularities in the estrous cycle may occur and fertility is adversely affected (Yolcu, 2024). Excess protein in the diet increases blood ammonia concentration and negatively affects fertility (Sonderman and Larson, 1989). According to our survey, 85.7% of owners believe that dietary protein level affects fertility, while 12.3% believe that dietary protein level does not affect fertility.

Mineral and vitamin deficiencies or excesses in ruminant diets affect fertility. According to our survey, 73.5% of farmers reported that dietary vitamin and mineral levels affect fertility, 18.4% reported that dietary vitamin and mineral levels do not affect fertility and 8.2% were undecided. While 67.3% of farmers added additional vitamin and mineral premixes to their rations, 30.6% did not add vitamin and mineral premixes and 2% reported that they sometimes added them. Although 73.5% of farmers believe

that vitamin and mineral levels in rations affect fertility, the fact that 67.3% of them add additional vitamin and mineral premixes to their rations may be due to economic problems in animal husbandry.

Mycotoxins that may occur after poor storage of feeds used in animal nutrition may affect fertility (Liu and Applegate, 2020). According to our survey, 93.9% of the farmers think that storage conditions will affect feed quality, while 6.1% think that they will not. 91.8% of the farmers stated that mold and toxins affect reproduction, 6.1% stated that mold and toxins do not affect reproduction, and 2% were undecided. In response to the question "Do you feed feed with mold to animals?" 89.8% of the farmers answered no, while 10.2% answered yes.

Pasture nutrition is very important in livestock production. It both reduces feed costs and has animal health benefits. However, fertility problems can also occur in pasture-fed animals. The main reason for this situation is the deterioration of existing pastures due to overgrazing and lack of necessary breeding work over time and the inability of animals to meet the nutrient needs for reproduction and productivity (Armağan and Işık, 2022). According to our survey, 10.2% of the farmers use pasture while 89.8% do not use pasture. In response to the question "Are the pastures sufficient in terms of animal nutrition", 89.8% of the farmers stated that the pastures are insufficient in terms of nutrition and additional nutrition is required. Among the farmers who feed their animals in the pasture, 80% of them use additional supplements to the pasture. These results clearly show that pastures are insufficient in terms of animal nutrition.

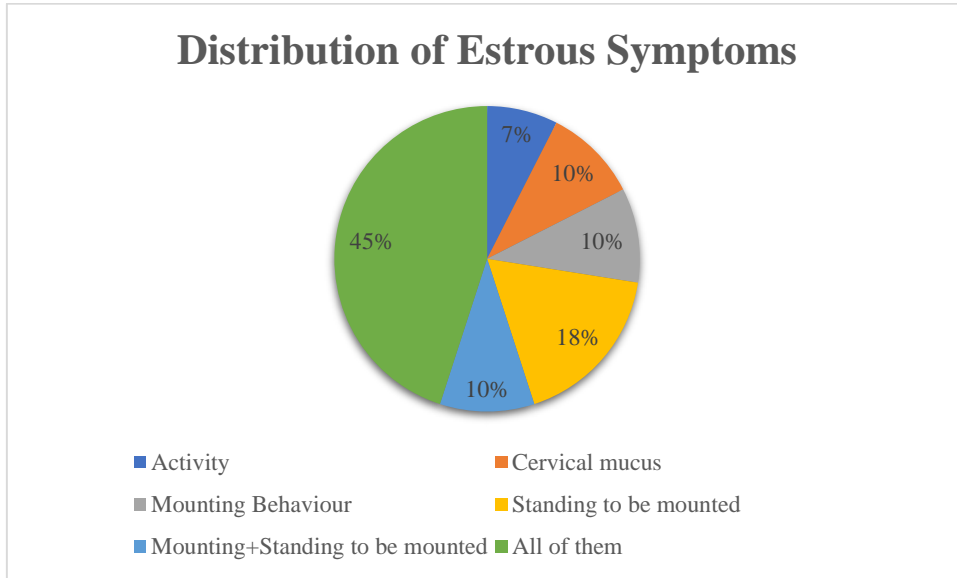
In response to the question "Are there any feeds that you add or remove from the ration to increase the pregnancy rate of cows", 93.9% of the farmers stated that they have not made any changes and 6.1% stated that they have made changes. To increase fertility, there are farmers who add carrots to the ration, add vitamin-mineral premix, add vetch, increase ration energy level and decrease ration alfa-alfa level. However, no clear success has been achieved.

The average calving interval obtained in the questionnaire study was 374.73 from 49 dairy cattle farms. It was determined that 67.34% of the dairy cattle farms were between 351-400 days. The average calving intervals obtained by Akman et al. (2011), İnci et al. (2007), Şahin and Ulutaş (2010), Yılmaz and Sarıözkan (2020) were 388.5, 383.1, 411.2, and 369.9 respectively. The average calving interval obtained in our study is similar to the researchers, but it was observed that the reproductive parameters exceeded the targeted 365 days.

As a result of the survey, the duration of days open was found to be 95.61 days. Considering that the target value should not exceed 90 days, the days open are not within the target range. However, considering other studies, it is reported that the average number of days open of active dairy farms in the survey study conducted by Yılmaz and Sarıözkan (2020) was 82.9 days, and the number of days open in the studies conducted by İnci et al. (2007) and Şahin and Ulutaş (2010) was 135.8 and 99.5 days, respectively. Therefore, the value of days open we obtained is shorter compared to the dairy farms of Polatlı and Altınova. In our study, it was observed that the average duration of the first postpartum estrus was 44.30 days and 71.42% of the dairy cattle farms were between 20-45 days. Considering that the target value should not exceed 40 days and the service period is shorter compared to the dairy cattle farms in other regions, it is concluded that the dairy cattle farms in Ereğli district are doing well in the postpartum estrus follow-up and the follow-up of the postpartum process. In the study conducted by Şahin and Ulutaş (2010), the average age at first insemination was reported to be around 18 months. This situation is similar to the fact that dairy farms in Ereğli district prefer 14-18 months as the age of first mating/insemination. Yılmaz and Sarıözkan (2020) stated that the semi-open type of 3 dairy cattle farms, which they found to be effective in their survey study, can increase the efficiency in terms of

reproductive parameters. In the survey we conducted in Ereğli district, although 87.8% of the 49 dairy cattle farms were semi-open, it was observed that the average of artificial insemination per pregnancy was 2.27 and the average of natural mating per pregnancy was 1.7. Both parameters are not within the target values. The pregnancy rate can also be influenced by various factors such as the correct timing of insemination and regular estrus monitoring. Considering the estrous symptoms and distributions shown in the Figure observed by the breeders in our study, it can be assumed that the breeders are relatively aware.

Figure Distribution of estrous symptoms observed in dairy cattle farms



CONCLUSION

In conclusion, it was found that dairy cattle farms in Ereğli district of Konya province were not effective in terms of reproductive parameters evaluated in the study. Since some parameters are close to the target values, it is assumed that adequate conditions are provided in terms of prenatal and postnatal care and necessary follow-ups are carried out. It is also observed that the breeders in the region are aware of the nutritional conditions. However, it is believed that the desired pregnancy rates can be achieved by improving the nutritional conditions. These results on grazing clearly show that pastures are inadequate in terms of animal nutrition. It is believed that the presented survey study will be useful for researchers as it carries information about regional data of Ereğli district of Konya province.

Ethical Statement

This study is not based on the any master's/doctoral thesis.

This article was not produced by developing and partially modifying the content of the paper presented orally at any symposium, and no full text was published.

Ethics Committee Approval

28/03/2024 dated and 2024/063 numbered was given by Selçuk University, Faculty of Veterinary Medicine Experimental Animal Production and Research Center Local Ethics Committee

Funding

The researchers did not receive funding from any institution or organization for this research.

Conflict of Interest

All authors declare that they have no conflicts of interest.

Author Contributions

Research Design (CRediT 1) Author 1 (%33,3) – Author 2 (%33,3) – Author 3 (%33,3)

Data Collection (CRediT 2) Author 1 (%45) – Author 2 (%55)

Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%33,3) – Author 2 (%33,3) – Author 3 (%33,3)

Writing the Article (CRediT 12-13) Author 1 (%40) – Author 2 (%40) – Author 3 (%20)

Revision and Improvement of the Text (CRediT 14) Author 1 (%40) – Author 2 (%40) – Author 3 (%20)

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Effects of Doxorubicin Administration at Different Doses and Durations on the Body Weight of Rats

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Article Info

Received: 11.09.2024
Accepted: 26.10.2024
Online First: 04.12.2024
Published: 23.01.2025

Keywords:

Body weight loss,
Cachexia,
Doxorubicin.

ABSTRACT

This study examines the impact of varying doses of Doxorubicin (DOX) on body weight in rats, with a focus on the drug's cachexia-inducing properties. While DOX is a widely used anticancer drug, it is associated with significant side effects, including cardiotoxicity and cachexia, which occur through mechanisms such as reduced insulin sensitivity, impaired glucose uptake, and decreased adipogenesis. In this study, 12-week-old male Wistar albino rats were divided into four groups: Control (C), low-dose DOX (DOX-L, 3 mg/kg), high-dose DOX (DOX-H, 12 mg/kg), and cumulative-dose DOX (DOX-C, 3 mg/kg every 24 hours for 4 days). The rats' weights were measured after administering various doses of DOX. Results indicated that the DOX-H group, which received a single high dose of DOX, experienced greater body weight loss compared to the other groups. The DOX-C group, which received the same total dose of DOX in cumulative doses, showed less weight loss compared to the DOX-H group. This suggests that single high-dose DOX applications lead to more significant body weight loss than cumulative dosing. The similarity in body weight loss between the DOX-L group (receiving a single low dose) and the control group indicates that DOX dosage is more influential than exposure duration. Given the potential for DOX to exacerbate cachexia in cancer patients, it is concluded that administering DOX in lower or cumulative doses may be preferable to a single high dose. In light of this information, we recommend further studies to determine the optimal dosage and duration of DOX administration, and to explore potential protective agents that could reduce DOX-related side effects on treatment.

To cite this article:

Kenar Çelik, Z., & Oruç, E. (2025) Effects of Doxorubicin Administration at Different Doses and Durations on the Body Weight of Rats. *Research and Practice in Veterinary and Animal Science (REPVAS)*, 2(1), 49-54. <https://doi.org/10.69990/REPVAS.2025.2.1.5>

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INTRODUCTION

Chemotherapy administration, while being a potentially curative or survival-extending treatment for many types of cancer, can also lead to severe cachexia and sarcopenia (Evans et al., 2008; Fearon et al., 2011; Cella et al., 2024). Daly et al. (2018) reported in their study involving 225 patients that chemotherapeutic agents used during cancer treatment exacerbate symptoms such as cancer-related cachexia, weakness, and sarcopenia. Cachexia alone accounts for 20% of cancer-related deaths (Ni and Zhang, 2020; Cella et al., 2024). This has led to a need for further research into cachexia induced by chemotherapeutic agents. Doxorubicin (DOX) is a widely used anthracycline derivative in the treatment of various cancers and continues to be employed as an anticancer drug (Schirone et al., 2022). DOX exerts its effects through topoisomerase II, which is involved in DNA replication, and also induces cell death through the production and accumulation of reactive oxygen species within the cell (Rawat et al., 2021). Despite being an effective antineoplastic agent, DOX has had its use restricted since its early years due to its cardiotoxicity (Bachur, 1979). In addition to its cardiotoxic effects, DOX, like many chemotherapeutic agents, also induces cachexia in the organism (Panjrath et al., 2007; Xiang et al., 2009; Arunachalam et al., 2012; Cella et al., 2024; Pandey et al., 2024). It has been reported that DOX induces this effect through mechanisms such as reduced insulin sensitivity, decreased adipogenesis, triggering of hyperglycemia, decreased glucose uptake by cells, and inhibition of lipolysis (Biondo et al., 2016). Literature searches have not revealed sufficient studies on changes in body weight of experimental animals subjected to DOX at various doses and durations. This study was conducted to elucidate the changes in body weight observed in rats subjected to low and high doses of DOX, as well as high-dose treatments administered in divided days.

MATERIALS and METHODS

For the purpose of the study, 24 male Wistar albino rats, each 12 weeks old, were obtained from the Experimental Medicine Research and Application Center of Selçuk University. The rats were randomly divided into four groups, each consisting of six rats (Control-C, low-dose DOX-DOX-L, high-dose DOX-DOX-H, and cumulative-dose DOX-DOX-C), and their weights were recorded using a precision balance. Throughout the study, the rats were provided with ad libitum access to food, unlimited water, and a lighting schedule of 12 hours light and 12 hours dark, with a temperature maintained at $22\pm 3^{\circ}\text{C}$. In the study, the control group received a single dose of 12 ml/kg saline intraperitoneally (ip), the DOX-L group received a single dose of 3 mg/kg DOX ip, the DOX-H group received a single dose of 12 mg/kg DOX ip, and the DOX-C group received 3 mg/kg DOX ip administered four times at 24-hour intervals (a total of 12 mg/kg). On the 7th day after the final DOX injection, the experimental animals were weighed again using a precision balance, and the results were recorded.

Statistical Analysis

The parametric data obtained from the measurements were analyzed using SPSS 22.0 statistical software, employing One-Way ANOVA followed by post hoc Duncan's test. Results are presented as mean \pm standard error (mean \pm SE). A p-value of < 0.05 was considered statistically significant.

RESULTS

The body weight values obtained from weighing the animals at the beginning of the experiment and prior to necropsy, along with the proportional differences and statistical values, are presented in Tables 1-2 and Figure.

Table 1. The weights of the animals used in the experiment, measured in grams (g) at the beginning (initial) and at the end (final) of the study.

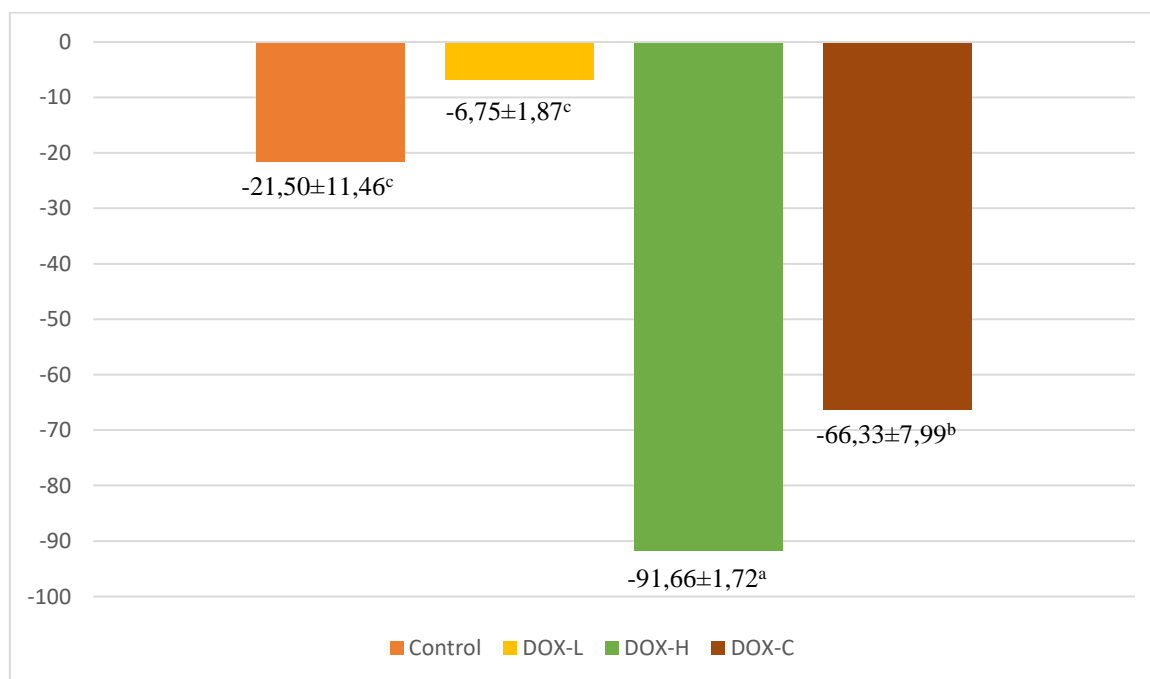
	C	C	DOX-L	DOX-L	DOX-H	DOX-H	DOX-C	DOX-C
	initial	final	initial	final	initial	final	initial	final
1	332	332	445	444	411	316	380	316
2	390	342	438	434	378	288	374	314
3	364	336	347,5	336	353,5	266	338	292
4	473	494	348	342	357,5	270	354	292
5	330	308	433	420	362	270	362	300
6	300	248	277	272	326	228	322	218

Table 2. The mean differences (in grams) between the pre-experiment and post-experiment weights of the rats, categorized by group.

Groups	Control	DOX-L	DOX-H	DOX-C
Body weight difference	-21,50±11,46 ^c	-6,75±1,87 ^c	-91,66±1,72 ^a	-66,33±7,99 ^b

Values within a row that do not share a common letter (a, b, c) are significantly different according to One-Way ANOVA and post-hoc Duncan's test ($p < 0.001$). Results are presented as mean ± SE

Figure. Graphical representation of the statistics for the differences between the initial and final weights of the rats.



DISCUSSION

In addition to their cancer-treating effects, chemotherapeutics are known to exacerbate cancer-related cachexia (Daly et al., 2018). During DOX treatment, cachexia is induced through mechanisms such as reduced insulin sensitivity, decreased adipogenesis, and inhibition of lipolysis (Biondo et al., 2016). In the conducted study, an evaluation of body weight changes in rats after administration of different doses and durations of DOX revealed that the DOX-H group, which received a single high dose of DOX, experienced a greater loss of body weight compared to other groups, with the DOX-C group following. These results suggest that a single high dose of DOX causes more significant body weight loss than cumulative DOX administration. Biondo et al. (2016) administered a single dose of 15 mg/kg DOX to 26 rats and performed euthanasia 72 hours post-administration to examine their adipose tissues. They also assessed parameters such as glucose uptake, adipogenesis, and lipogenesis in cell cultures following DOX administration. Their findings indicated that DOX caused weight loss in rats compared to the control group and negatively affected glucose uptake, adipogenesis, lipogenesis, and lipolysis in both in-vitro and in-vivo trials. Kelishomi et al. (2008) administered a total of 20 mg/kg DOX to rats over four weeks, with weekly injections, and reported a decrease in body weight due to DOX. In our study, the greater weight loss observed in the DOX-H group compared to other groups suggests that a single high dose of DOX has a more pronounced negative impact on body weight. The fact that the DOX-C group, which received the same total amount of DOX as the DOX-H group but in cumulative doses, experienced less weight loss compared to the DOX-H group supports this notion. Although the weight loss in the DOX-C group was not as severe as in the DOX-H group, it was significantly higher than that in the control and DOX-L groups, highlighting the effect of DOX dosage on body weight loss. Interestingly, despite statistical insignificance, the weight loss in the DOX-L group was found to be less than in the control group. The single 3 mg/kg dose of DOX administered to the DOX-L group resulted in an average weight loss of 6.75 grams, underscoring the importance of DOX dosage on body weight. Although the conditions were controlled and consistent, the weight loss observed in the control group may be attributed to individual stress factors.

CONCLUSION

The body weight loss observed in the DOX-H group, which received a single 12 mg/kg dose of DOX, was more severe compared to the other groups. In contrast, the DOX-C group, which received a cumulative dose of 12 mg/kg DOX, experienced significant body weight loss, though not as pronounced as that in the DOX-H group. The statistical similarity between the DOX-L group, which received a single 3 mg/kg dose, and the control group suggests that the dose of DOX is more influential on body weight than the exposure duration. Given that cachexia accounts for approximately 20% of cancer-related deaths and the potential for chemotherapeutics used in cancer treatment to exacerbate cachexia, it is concluded that administering DOX in lower or cumulative doses might be more appropriate than a single high dose. However, to determine the optimal application method and dose, further studies are recommended to investigate the effects of DOX at different durations and doses on body weight, as well as to explore protective agents that might mitigate the adverse effects of DOX on body weight.

Ethical approval

22/11/2021 dated and numbered 2021/60 was given by Selcuk University Experimental Medicine Application and Research Center ethics committee.

Ethical Statement

This study is based on the doctoral thesis entitled “Investigation of Heart Tissue Damage in Doxorubicin-Administered Rats by Molecular and Pathological Methods”, submitted under the supervision of “Thesis defense committee” on 27.06.2024 date.

Author Contributions

Research Design (CRediT 1) Author 1 (%50) – Author 2 (%50)

Data Collection (CRediT 2) Author 1 (%50) – Author 2 (%50)

Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%50) – Author 2 (%50)

Writing the Article (CRediT 12-13) Author 1 (%50) – Author 2 (%50)

Revision and Improvement of the Text (CRediT 14) Author 1 (%50) – Author 2 (%50)

Funding

This study was supported by the Selcuk University Scientific Research Projects Coordination with the number 21112009.

Conflict of Interest

The authors declare that there is no conflict of interest between the authors.

Acknowledgements

This study is based on the doctoral thesis entitled “Investigation of Heart Tissue Damage in Doxorubicin-Administered Rats by Molecular and Pathological Methods”, submitted under the supervision of “Thesis defense committee” on 27.06.2024 date.

Sustainable Development Goals (SDG)

3 Good Health and Well-Being,

15 Life on Land.

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The Effect of Body Condition Scoring on Lamb Development in Akkaraman and Lalahan Genotypes

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Article Info

ABSTRACT

Article History

Received: 21.05.2024

Accepted: 05.11.2024

Online First: 04.12.2024

Published: 23.01.2025

Keywords:

Akkaraman sheep,
Condition score,
Growth characteristics,
Kıvırcık x Akkaraman G1

The study was conducted at the International Livestock Research and Training Center Directorate and investigated the effects of ewes' body condition score (BCS) on certain body measurements of their lambs at birth and at 3 months of age in Akkaraman and Lalahan (Kıvırcık x Akkaraman G1 crossbred) sheep in Ankara province. The animal material consisted of 106 lambs (Akkaraman = 33, Lalahan = 73) at birth and 144 lambs (Akkaraman = 33, Lalahan = 111) at 3 months, along with their dams. The BCS values of the ewes were divided into three groups for analysis: low (≤ 2.5), medium (3.0), and high (≥ 3.5). The general means of BCS values of the ewes at birth and at 3 months were found to be 2.86 and 2.84, respectively. The effects of genotype and dam age on BCS were not significant in either period. The live weight (LW), wither height, rump height, chest depth, body length, and chest girth values of the lambs were 4.8 kg, 42.1 cm, 42.6 cm, 16.6 cm, 33.0 cm, and 38.5 cm at birth, and 24.0 kg, 62.2 cm, 63.0 cm, 27.8 cm, 58.8 cm, and 65.3 cm at 3 months of age, respectively. Ewes with high BCS had heavier LW lambs at both birth (low: 4.5 kg, medium: 4.8 kg, high: 4.9 kg; $P < 0.05$) and 3 months (low: 24.3 kg, medium: 25.1 kg, high: 25.8 kg; $P > 0.05$). A similar pattern was observed in the daily live weight gain (DLWG) of lambs (low: 0.205 kg, medium: 0.221 kg, high: 0.242 kg; $P < 0.001$). In conclusion, it can be said that ewes with a high BCS adequately meet their nutritional needs during lactation, thereby providing better care for their lambs.

To cite this article:

Firdolaş, S., Sakar, Ç.M., Cöner, F.I., Aydın, A.A., & Erat, S. (2025) The Effect of Body Condition Scoring on Lamb Development in Akkaraman and Lalahan Genotypes. *Research and Practice in Veterinary and Animal Science (REPVAS)*, 2 (1), 55-65.

<https://doi.org/10.69990/REPVAS.2025.2.1.6>

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INTRODUCTION

In sheep breeding, knowing the body condition of sheep during different stages of the production cycle is crucial (Koyuncu et al., 2018). The condition affects the performance of animals at any stage, and body condition score (BCS) is a method used to rate the levels of fat deposition in the organism based on physical characteristics (Sarı et al., 2013). The fastest and cheapest way to determine the condition score is to assess the general state and some reference points (shoulder, buttocks, back, tail head, and chest) visually and by touch (Kor and Ertuğrul, 2000). There is an optimal BCS for each stage of the production cycle of sheep in the flock (Koyuncu et al., 2018). Sheep with different BCSs during periods such as reproduction, pregnancy, lambing, and lactation should be subjected to specific feeding regimens based on these scores (Şireli, 2019). The body condition during breeding and lambing directly affects the performance and productivity of both sheep and their lambs (Karakuş and Atmaca, 2016). During the lambing period, the critical lower limit of the condition score is around 2.5, and sheep above this score are less affected by nutritional deficiencies that may occur in the first weeks of lactation (Sarı et al., 2013).

Animal-derived foods play an important role in human nutrition, as they contain essential proteins, vitamins, and minerals necessary for human health (Oyan et al., 2024). Therefore, it is important to enhance fertility in sheep and support the healthy lives of lambs after birth. In most sheep production systems practiced under natural grazing conditions, sheep mobilize their body reserves to overcome periods of feed scarcity (Sezenler et al., 2011). Regular monitoring of the condition score ensures the maintenance of a healthy and productive flock and provides information about the animals' nutritional and health status (Koyuncu et al., 2018). Good body condition is particularly critical in late pregnancy and the lambing period for quality colostrum production and milk yield (Karakuş and Atmaca, 2016).

Sheep breeding is closely associated with the use of pasture and fallow lands, which are vital for nutrition, employment, rural development, and sociology worldwide. Sheep receive more attention than cattle due to their shorter gestation periods, higher twinning rates, lower slaughter age, and better utilization of roughage (Uysal et al., 2024). Sheep constitute a significant part of the livestock sector in Türkiye, making up about 60% of domestic animals (FAO, 2022). The majority of sheep bred in Türkiye are native breeds, with the Akkaraman breed, which accounts for 40-45% of the small ruminant population, being the most common in Central Anatolia (Şahin, 2023; Sakar, 2024). The Akkaraman sheep is a fat-tailed breed adapted to the region's harsh climatic conditions, raised for meat and milk production (Figure 1a). Lalahan sheep (Kıvrıkcık: 0.75 x Akkaraman:0.25, G1) is a genotype developed at the Lalahan International Livestock Research and Training Center (Ankara) for obtaining a new genotype suitable for steppe region conditions for lamb meat production (Erol et al., 2017). This type has a white fleece-covered body with black or brown spots on the head, face, and ears (Figure 1b).

Figure 1. Akkaraman sheep (a), Lalahan genotype (b)



This study aims to examine the effects of body condition scores of the Akkaraman and Lalahan genotype ewes during birth and 3 months of age on the growth and development of their lambs. The study evaluates the BCS of the dams at birth and at 3 months, aiming to reveal the relationship between these values and lamb birth weight as well as some body measurements.

MATERIAL and METHOD

Animal Material

The study was conducted at the International Livestock Research and Training Center Directorate (ILRTC). The animal material consisted of Akkaraman and Lalahan (Kıvrıcık x Akkaraman G1) lambs born in February-March 2021, along with their dams. Body measurements were taken from a total of 106 lambs (Akkaraman: 33 head, Lalahan: 73 head) during the birth period and from 144 lambs (Akkaraman: 33 head, Lalahan: 111 head) at 3 months.

Mating was carried out in September and lasted about a month. Most births occurred in February. The animals were taken to pasture from April to October, with no additional feed provided during this period. During the winter, animals were given a diet of 40% roughage and 60% concentrate feed. Alfalfa hay and barley straw were provided as roughage. The ewes were supplemented with concentrate feed two weeks before the breeding season. In the last three weeks of pregnancy, the ewes were given 700 g/head/day of concentrate feed, and at the beginning of lactation, they received 400 g/head/day. Feeding was done twice daily at 08:30 a.m. and 4:30 p.m.

Lambs were kept with their dams in individual pens for 1-2 days after birth. They were allowed to suckle freely while remaining continuously with their dams. No weaning program was implemented during the suckling period. The animals were then taken to pasture with their dams, with no additional feeding provided during this period.

Data Set

All animals were regularly recorded in the farm registry with information such as birth date, sex, dam number, and age. Additionally, lambs were recorded by attaching ear tags within 1-2 days after birth. Measurements were taken at birth (BM) and at 3 months (3M). During these two periods, the live weight (LW), withers height (WH), rump height (RH), chest depth (CD), body length (BL), and chest girth (CG) of the lambs were recorded. The weights were measured using a scale (Iconix FX41) with an accuracy of 50 g. Other measurements were taken using a measuring stick and tape measure. While the lambs' birth measurements were taken within the first 24 hours after birth, the 3-month measurements were taken within ± 2 days of reaching 90 days of age. Additionally, the average daily weight gain (DWG) of lambs between birth and 3 months was calculated using the formula (kg/day): $(3 \text{ months LW} - \text{birth weight}) / (\text{date of 3 months LW measurement} - \text{birth date})$.

In addition, the Body Condition Score (BCS) of the dams of the lambs was recorded during the birth and 3-month periods. BCS values of the ewes were measured shortly after birth. These values were recorded on a scale ranging from 1 to 5 points, in intervals of 0.5 (Russell et al., 1969). Two referees simultaneously scored the BCS values.

In the case of discrepancies between the referees, assistance was sought from a third expert, and the score given by that expert was considered the final score. If differences persisted in the independently determined BCS values, scoring continued until a consensus was reached among the referees. In the study, BCS values were divided into three groups: 1) low: ≤ 2.5 , 2) medium: 3.0, and 3) high: ≥ 3.5 (Koyuncu et al., 2018).

Statistical Analysis

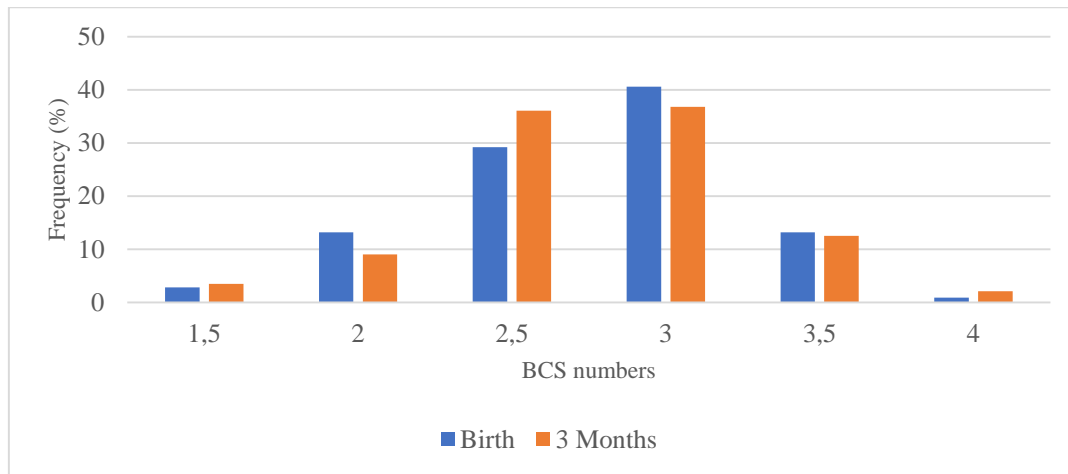
All body measurement values were analyzed using the General Linear Model (GLM) procedure. Average differences between groups were tested with Tukey’s Multiple Comparisons. Data analysis was conducted using Minitab Statistical Software (Minitab, 2010). The GLM formula is as follows;

$Y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + f_m + e_{ijklmn}$. Where; Y_{ijklmn} : observed data; μ : Overall mean; a_i : i. effect of BCS (1: low (≤ 2.5), 2: medium (3.0), 3: high (≥ 3.5)); b_j : j. effect of genotype (1: Akkaraman, 2: Lalahan); c_k : k. effect of sex (1:female, 2: male); d_l : l. effect of birth type (1:single, 2:twin); f_m : m. effect of dam age (2-3, 4-6, 7+); e_{ijklmn} : random error.

RESULTS

In the study, examinations were conducted on 106 lambs at birth and 144 lambs at the 3-month period, along with their dams, from the Akkaraman and Lalahan genotypes. The percentage distribution of the BCS values observed in sheep at birth and 3 months is shown in Figure 2. In the study, BCS values below 1.5 and above 4 were not encountered in either period. At birth, the most common BCS value observed in sheep was 3 (40.6%), followed closely by a BCS score of 2.5 (29.2%). At 3 months, the most common BCS values were again 3 (36.8%) and 2.5 (36.1%), with these values being close to each other.

Figure 2. The frequency distribution of BCS in ewes



In the study, the BCS values obtained from the dams during the birth and 3-month periods are presented in Table 1. These values were 2.86 during the birth period and 2.84 during the 3-month period. The BCS values were found to be statistically not significant between genotypes and age groups of the dams in both the birth and 3-month periods.

Table 1. BCS values of the dam.

Factors	Birth		3 Months	
	n	BCS	n	BCS
Overall		2.86±0.051		2.84±0.049
Genotype		NS		NS
Akkaraman	33	2.91±0.083	33	2.85±0.085
Lalahan	73	2.82±0.058	111	2.84±0.047
Dam age		NS		NS
2-3	42	2.87±0.078	53	2.91±0.072
4-6	30	2.89±0.088	53	2.87±0.072
7+	34	2.83±0.087	38	2.74±0.085

NS: non-significant

The values of the body measurements taken from the lambs during the birth period, according to environmental factors are presented in Table 2. During the birth period, the values for BW, WH, RH, CD, BL, and CG were generally found to be 4.8 kg, 42.1 cm, 42.6 cm, 16.6 cm, 33.0 cm, and 38.5 cm, respectively. According to the BCS values of the dams, the BW, WH, and RH values were higher in lambs born from high-BCS dams, while CD, BL, and CG values were higher in lambs born from medium-BCS dams. The differences between the groups were significant ($P<0.05$) for BW, WH, RH and CD values, but not significant for the others. The measurement values for Akkaraman genotype lambs were higher than those for Lalahan genotype lambs in all parameters, with significant differences ($P<0.05$) in WH, RH, CD, and BL values, while the differences in BW and CG values were found to be not significant.

Table 2. The least squares mean and standard errors of body measurements in the birth period

Factors	n	LW (kg)	WH (cm)	RH (cm)	CD (cm)	BL (cm)	CG (cm)
Overall	106	4.8±0.108	42.1±0.271	42.6±0.295	16.6±0.593	33.0±0.293	38.5±0.324
Dam BCS		*	*	**	*	NS	NS
Low (≤ 2.5)	48	4.5±0.126 ^b	41.5±0.327 ^b	41.6±0.345 ^b	16.0±0.698 ^b	32.9±0.338	38.3±0.376
Medium(3.0)	43	4.8±0.126 ^a	42.1±0.337 ^{ab}	42.8±0.356 ^{ab}	17.8±0.704 ^a	33.5±0.352	38.7±0.389
High (≥ 3.5)	15	4.9±0.193 ^a	42.7±0.518 ^a	43.3±0.542 ^a	15.9±1.118 ^b	32.7±0.547	38.6±0.598
Genotype		NS	***	***	*	**	NS
Akkaraman	33	4.8±0.152	42.8±0.388 ^a	43.3±0.422 ^a	17.4±0.895 ^a	33.6±0.418 ^a	38.7±0.464
Lalahan	73	4.7±0.106	41.4±0.267 ^b	41.8±0.291 ^b	15.7±0.600 ^b	32.4±0.290 ^b	38.4±0.319
Sex		NS	**	**	NS	***	NS
Birth type		***	NS	NS	NS	NS	**
Dam age		*	NS	NS	NS	*	NS

^{a,b,c} Values within a column with different superscripts differ significantly at $P<0.05$. ***: $P<0.001$, **: $P<0.01$, *: $P<0.05$, NS: non-significant. LW: live weight, WH: withers height, RH: rump height, CD: chest depth, BL: body length, CG: chest girth

The values of the body measurements taken from the lambs at 3 months, according to environmental factors, are presented in Table 3. All values were found to be higher in lambs born to high-BCS dams, however, the differences between the groups were not significant for all measurements. Additionally, when differences by genotype were examined, BW, WH, and RH values were significant

($P < 0.001$), while the other values were found to be not significant. For all measurements, Akkaraman lambs were found to have higher values than Lalahan lambs.

Table 3. The least squares mean and standard errors of body measurements in the 3 months and DWG

Factors	n	LW (kg)	WH (cm)	RH (cm)	CD (cm)	BL (cm)	CG (cm)	DWG (kg)
Overall	144	24.0±0.453	62.2±0.450	63.0±0.507	27.8±0.318	58.8±0.555	65.3±0.587	0.222±0.0054
Dam BCS		NS	NS	NS	NS	NS	NS	***
Low (≤ 2.5)	70	24.3±0.495	61.7±0.486	62.6±0.547	27.6±0.343	58.5±0.597	64.3±0.635	0.205±0.0064 ^b
Medium (3.0)	53	25.1±0.579	62.1±0.581	62.7±0.654	27.8±0.409	58.3±0.708	65.1±0.751	0.221±0.0064 ^{ab}
High (≥ 3.5)	21	25.8±0.813	63.0±0.833	63.8±0.938	28.1±0.586	58.6±1.026	66.5±1.083	0.242±0.0093 ^a
Genotype		***	***	***	NS	NS	NS	***
Akkaraman	33	26.7±0.536 ^a	63.7±0.703 ^a	64.5±0.792 ^a	28.1±0.497 ^a	58.9±0.867 ^a	65.3±0.917 ^a	0.239±0.0074 ^a
Lalahan	111	23.4±0.544 ^b	60.8±0.407 ^b	61.5±0.458 ^b	27.6±0.286 ^b	58.7±0.498 ^b	65.2±0.526 ^b	0.206±0.0055 ^b
Sex		***	***	***	NS	NS	NS	**
Birth type		***	***	***	***	***	***	***
Dam age		NS	NS	NS	NS	NS	NS	NS

^{a,b,c} Values within a column with different superscripts differ significantly at $P < 0.05$. ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, NS: non-significant. LW: live weight, WH: withers height, RH: rump height, CD: chest depth, BL: body length, CG: chest girth, DWG: average daily weight gain

The DWG values of the lambs between birth and 3 months are presented in Table 3. These values were generally found to be 0.222 kg. The effect of the dam's BCS at birth on the DWG values was found to be significant ($P < 0.001$), with these values being 0.242 kg for the high group, 0.221 kg for the medium group, and 0.205 kg for the low group, in descending order. This value was also found to be higher in the Akkaraman genotype (0.239 kg) compared to the Lalahan genotype (0.206 kg) ($P < 0.001$).

DISCUSSION

In the study, the BCS values obtained from the ewes at both periods (birth and 3 months) were found to be similar across genotypes and age groups (Table 1). This could be due to the uniform management practices on the farm, where all sheep are kept together and subjected to the same care, feeding, and grazing conditions. Similar BCS values were reported in the birth period for the Norduz breed at 2.9 (Karakuş and Atmaca, 2016), the Kıvırcık breed at 2.9 (Koyuncu et al., 2018), and the dairy breed Awassi at 2.64 (Şireli, 2019). The findings from this study were similar to those of meat or dual-purpose breeds but higher than those of dairy breeds.

While the birth weight averages of Akkaraman lambs with low, medium, and high dam BCS values at birth were determined as 4.5, 4.8, and 4.9 kg, respectively, the differences were found to be significant ($P < 0.05$; Table 2). In Tuj sheep, the birth weights of lambs were 3.46, 3.76, and 3.85 kg in the ≤ 2 , 2.5-3.5, and ≥ 4 dam BCS groups, respectively ($P < 0.01$), with the effect of sex being not significant, but the effects of birth type and ewe age being significant ($P < 0.01$) (Sarı et al., 2013). In a study with Karacabey Merino sheep, the average birth weights of lambs born to ewes with BCS values of ≤ 2 , 3, and ≥ 4 at birth were found to be 4.38, 4.74, and 5.03 kg, respectively ($P < 0.05$), with the effect of dam age also being significant ($P < 0.05$) (Sezenler et al., 2008). In Kıvırcık sheep, the birth weights of lambs in the ≤ 2.5 , 3, and ≥ 3.5 ewe BCS groups were reported as 4.0, 4.3, and 4.5 kg, respectively ($P < 0.01$). The effects of sex and birth type on birth weight were also significant ($P < 0.01$) (Koyuncu et al., 2018). In the Lori-Bakhtiari breed, the highest lamb birth weights were found in lambs from ewes with BCS values of 3.5, 3.0, 2.5, 4, 1, and 2, respectively ($P < 0.01$), with the effect of sex also being significant (Vatankhah et al., 2012). In Sanjabi sheep, it was reported that the birth weight of lambs was significantly affected ($P < 0.05$) by the ewe's BCS, with ewes having a BCS of 3 giving birth to lambs

with higher live weights than those in other groups (Jalilian and Moeini, 2013). In Afshari sheep, it was reported that reproductive performance was better and lamb birth weight was higher in ewes with a BCS of 3 (Aliyari et al., 2012).

In contrast to the literature information reported above, there are also studies indicating that dam BCS does not affect lamb birth weight. In a study with Türkgeldi sheep, it was reported that the effects of ewe condition score groups and birth type on lamb birth weight were not significant, while the effects of sex and dam age were significant ($P < 0.01$; $P < 0.05$) (Özder et al., 1997). In Norduz sheep, lamb birth weights from ewes with BCS values of 2.5, 3.0, and 3.5 were reported as 4.77, 4.92, and 5.18 kg, respectively ($P > 0.05$), with ewe age and sex also having no effect, while birth type had a significant effect ($P < 0.01$) (Karakuş and Atmaca, 2016). In Fat-tailed Barbarine sheep, it was determined that the BCS of ewes at lambing did not affect lamb birth weight ($P > 0.05$), with values of 3.8, 3.8, and 3.9 kg for the lean, medium, and fat groups, respectively (Yagoubi and Atti, 2020). Consistent with other studies, this study found that lambs from ewes with high BCS had significantly higher birth weights. However, in this study, the birth weight of lambs according to dam BCS and other environmental conditions was similar to some literature results but different from others. These differences are thought to be due to variations in genotype, the climatic and topographical geography of the region where the sheep are reared, and especially the nutrition of the ewe during pregnancy.

At 3 months old, inter-group differences in body measurement values of lambs based on dam BCS were found to be not significant; however, all values were higher in lambs born to high-BCS ewes. Condition scoring of ewes at lambing time and adjusting their nutrition based on their condition during the lamb-rearing period is beneficial for the development of lambs until weaning (Mathias-Davis et al., 2013). In Türkgeldi sheep, although significant differences were not found among the average weaning weights of the condition score groups determined during the lambing period, extreme values (1.5 and 3.5) were reported to have a negative effect on weaning weight (Özder et al., 1997). In Tuj sheep, the 90-day LW values of lambs were found in descending order for those in lambs born to ewes with ≥ 4 (20.37 kg), 2.5-3.5 (20.18 kg), and ≤ 2 (18.78 kg) BCS, respectively ($P > 0.05$), although the effect of birth type on this value was significant ($P < 0.001$), the effects of sex and ewe age were found to be not significant. (Sarı et al., 2013). In Norduz sheep, the 90-day LW values of lambs were found to be 26.16 kg, 24.45 kg, and 23.73 kg for ewes with BCS of 3.5, 3.0, and 2.5, respectively ($P > 0.05$), the effects of dam age (2, 3, 4), sex, and birth type on this value were also found to be not significant (Karakuş and Atmaca, 2016). In Kıvrıkcık sheep, the weaning weight of lambs born to ewes with BCS ≤ 2.5 , 3, and ≥ 3.5 was reported as 22.3, 23.0, and 23.1 kg, respectively ($P > 0.05$), the effects of sex and birth type on this value were significant ($P < 0.05$; $P < 0.01$) (Koyuncu et al., 2018). In this study, it was generally consistent with the literature findings that lambs with higher dam BCS at 90 days of age (or weaning age) had higher live weights.

In Sanjabi sheep, the effect of dam BCS on lamb weaning weight was found to be significant ($P < 0.05$), with the highest values reported in lambs from ewes with a BCS of 3 (24.2 kg) and > 3.5 (Jalilian and Moeini, 2013). Discrepancies in these values in the literature are reported to be due to differences in scoring timing, variations in the BCS scale, as well as differences in nutrition during the mid to late pregnancy and lactation periods (Karakuş and Atmaca, 2016). Although there was no significant difference in body measurements at 3 months, generally higher values were observed in lambs with high BCS, indicating better dam care. Higher BCS scores in ewes are associated with improved lamb growth, as reflected in other studies.

The effect of dam BCS on lamb DWG values was found to be significant ($P < 0.001$), with lambs from high BCS ewes having the highest DWG values (Table 2). DWG90 values in Karacabey Merino sheep were reported to be highest in lambs with a dam BCS of 3 (0.269 kg) ($P < 0.05$), along with

significant effects ($P<0.05$) of ewe age and sex (Sezenler et al., 2008). The BCS of Barbarine ewes at birth affected the live weight at 30 days, live weight at 70 days, and DWG of lambs ($P<0.01$), with better results reported from lambs born to ewes with a fat BCS (Yagoubi and Atti, 2020). In Tuj sheep, the highest value was found in lambs with a BCS of 2.5-3.5 (228.87 g) ($P>0.05$), while the effect of sex and birth type on this value was found to be significant ($P<0.01$; $P<0.05$), and ewe age had no significant effect (Sarı et al., 2013). A study with Kırıcık sheep reported that higher live weight and condition values positively affected lamb development, with lambs from ewes with a BCS ≥ 3.5 having the highest DWG90 value ($P>0.05$), and significant effects ($P<0.05$; $P<0.01$) of sex and birth type (Koyuncu et al., 2018). It has been explained that ewes with higher BCS can utilize body reserves to meet energy requirements for sustaining high milk production levels to nurse their lambs, even under inadequate nutrition conditions (Yagoubi and Atti, 2020). The differences between the findings of this study and some literature reports may arise from variations in breed, origin, ewe age, and differences in care and feeding practices. According to the findings obtained in this study, it can be said that high BCS ewes meet their nutritional needs during lactation adequately, thereby increasing both the quality and quantity of milk they provide to their offspring.

CONCLUSION

In this study, the relationship between the BCS of Akkaraman and Lalahan genotype ewes during the lambing and at 3 months, and the body measurements and DWG values of their lambs were investigated. It was found that lambs born to ewes with higher BCS had higher birth weights, and these differences were significant. Although no significant differences were found among groups in body measurements at 3 months, lambs born to ewes with generally higher BCS tended to have higher values. In terms of DWG values, lambs born to ewes with higher BCS showed better performance, suggesting that meeting the nutritional needs of ewes during lactation results in providing their offspring with higher quality and quantity of milk. Consequently, it was determined that the BCS of ewes during lambing and at 3 months has a significant impact on the growth and development of lambs. Therefore, closely monitoring the body condition scores of ewes and adjusting feeding strategies accordingly would be beneficial.

Acknowledgements

The authors would like to thank the “International Livestock Research and Training Center Directorate” for allowing the use of animal materials..

Ethics Committee Approval

27/08/2021 dated and numbered 192 was given by International Center for Livestock Research and Training ethics committee.

Authors Contributions

Research Design (CRediT 1) Author 1 (%40) - Author 2 (%30) - Author 5 (%30)

Data Collection (CRediT 2) Author 1 (%25) – Author 2 (%25) - Author 3 (%25) - Author 4 (%25)

Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%25) – Author 2 (%25) - Author 3 (%25) - Author 4 (%25)

Writing the Article (CRediT 12-13) Author 1 (%50) - Author 2 (%50)

Revision and Improvement of the Text (CRediT 14) Author 5 (%100)

Funding

There is not any funding in this study

Conflict of Interest

The authors declare that there is no conflict of interest.

Sustainable Development Goals (SDG)

Sustainable Development Goals: Does not support.

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